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NUTRITION REVIEWS

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NUTRITION REVIEWS

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HUMAN FITNESS AND THE STATE OF VITAMIN NUTRITION

Good nutrition has been defined in many ways. The challenging nature of the concept appears in the fact that many of the definitions are open to criticism on practical or theoretical grounds. One definition, which perhaps may be defended as well as any, is that good nutrition is that condition which permits the development and maintenance of the highest state of fitness (A. Keys, A. F. Henschel, O. Mickelsen, and J. M. Brozek, *J. Nutrition* **26,** *399 (1943)*). The onus then is shifted to the term "fitness," which is troublesome to define precisely because, to judge from its popularity in usage, everyone knows what it means but not how to measure it. Broadly, fitness embraces occupational work capacity, ability in physical and mental tasks, and power to withstand physical and biologic stresses. Even if there is arbitrary restriction to a particular aspect, such as "physical fitness," its evaluation is a complex and difficult problem (H. L. Taylor and J. Brozek, *Federation Proceedings* **3,** *216 (1944)*).

In any case, fitness connotes positive health as distinguished from disease so we are not here primarily concerned with the states of marked unfitness which occur in the classic vitamin deficiencies. It is pertinent, however, to examine the effects on fitness of milder deficiencies. Conversely, there is the problem of possible effects on fitness of unusually abundant vitamin intakes. Finally, there are the questions of the time course of alterations in fitness after changing the vitamin intake.

A major point at issue is whether fitness is peculiarly sensitive to differences in vitamin nutrition so that changes in fitness precede the development of other effects. Another question is concerned with the relative sensitivity of different aspects of fitness to dietary changes. These questions have special importance and it had been hoped that estimates of fitness might be helpful in recognizing the presence of vitamin deficiencies or, that differences in vitamin nutrition might help to explain differences in fitness of different individuals.

It is essential to recognize the limitations of current knowledge. Animal experiments contribute little to the present discussion. Nor, except in unusually well controlled experiments, can much reliance be placed on reports of the subjects themselves or the subjective impressions of observers. The number of acceptable investigations on vitamin-fitness relations is small and they have dealt with relatively short periods of time—from a few days to about a year—in the life of persons representing only a small part of the population; in the great majority of cases the subjects have been healthy males between 18 and 30 years of age. Finally, in evaluating the role of nutrition, it must be kept in mind that the term "fitness" has the restricted definition previously mentioned.

With these rather severe limitations in mind, the great bulk of the evidence indicates no gain in fitness of adults from vitamin supplementation added to a good American diet (A. Keys and A. F. Henschel, *J. Nutrition* **23,** *259 (1942)*; E. E. Foltz, A. C. Ivy, and C. J. Barborka, *J. Lab. Clin. Med.* **27,** *1396 (1942)*; J. W. Archdeacon and J. R. Murlin, *J. Nutrition* **28,** *241 (1944)*; J. M. Ruffin and D. Cayer, *J. Am. Med. Assn.* **126,** *823 (1944)*; R. E. Johnson, R. C. Darling, F. Sargent, and P. Robinson, *J. Nutrition* **29,** *155 (1945)*; H. Borsook *et al.*, *Milbank Memorial Fund Quart.* **24,** *99 (1946)*). There are opposing claims in the literature but most of these have so little basis as to deserve no separate mention here (*cf.*, A. Keys, *Federation Proceedings* **2,** *164 (1943)*). I. M. Frankau's claim (*Brit. Med.*

J. **2,** *601* (*1943*)) for a gain in physical fitness resulting from niacin supplementatior has not been confirmed. Incidentally, Frankau's test was not more rigorous than others as has been suggested (H. D. Kruse *et al.*, *Arch. Int. Med.* **74,** *258* (*1944*)). The weakness of the claim of R. F. Harrell for an improvement from thiamine supplementation has been discussed in this journal (*Nutrition Reviews* **4,** *343* (*1946*)).

Theoretically, it could be suggested that vitamin supplementation might improve fitness in some environments even though exerting no effect under ordinary conditions. In effect, this would suggest that vitamin "requirements" might be affected by the physical environment. The few experiments conducted so far have given no support to claims that supplementary vitamins increase fitness in hot weather (*Nutrition Reviews* **2,** *219* (*1944*); A. Henschel *et al.*, *Am. J. Trop. Med.* **24,** *259* (*1944*)). Much the same seems to be the case in cold environments although only one major experiment has been reported (N. Glickman, R. W. Keeton, H. H. Mitchell, and M. K. Fahnestock, *Am. J. Physiol.* **146,** *538* (*1946*)).

Evidence is gradually accumulating regarding the time course of events following a change from a fairly good diet to one definitely low in vitamins. Changes in physical fitness are neither early nor especially prominent results of diets extremely deficient in vitamin A (G. Wald, L. Brouha, and R. E. Johnson, *Am. J. Physiol.* **137,** *551* (*1943*)); Vitamin A Subcommittee, *Nature* **156,** *11* (*1945*)). Subsistence on a diet very low in ascorbic acid eventually produces a decline in physical fitness but this does not seem to precede the more characteristic changes in the gums, capillary resistance, and wound healing (*cf.* A. Keys, *Federation Proceedings* **2,** *164* (*1943*)). With both vitamin A and ascorbic acid it is not proper, from the present evidence, to suggest that low states of fitness may be ascribed, in the absence of other signs, to deficiencies in these vitamins.

The greatest interest regarding fitness has centered on the vitamins of the B-complex. The participation of some of the B-vitamins in energy yielding reactions might suggest an early decline in physical capacity when the supply of these vitamins is reduced. Actually, it seems that some other effects are more readily detected.

On diets in which the major B-vitamins are reduced to 34 to 40 per cent of the National Research Council's Recommended Daily Allowances, elaborate and well controlled studies have failed to disclose any loss in physical or mental capacity within periods up to at least six months (A. Keys *et al.*, *Am. J. Physiol.* **144,** *5* (*1945*); R. C. Cogswell *et al.*, *Ibid.* **147,** *39* (*1946*)). When these vitamins are reduced to extremely low levels in the diet, the first notable effects, which may appear in a week or two, are anorexia and mental depression; some of the subsequent loss of fitness may be due to plain semistarvation (A. Keys *et al.*, *op. cit.* (*1945*)). After the effects from general deprivation of B-vitamins are well established, the provision of thiamine alone tends to correct the situation, indicating that under these conditions thiamine is more critical than the other B-vitamins. It is significant that, so far, clearly positive results have been obtained only when thiamine has been restricted; no changes in fitness were observed in six months on a diet providing only 0.31 mg. of riboflavin per 1000 calories (A. Keys *et al.*, *J. Nutrition* **27,** *165* (*1944*)).

The several aspects of fitness are not equally responsive to acute restriction of the B-vitamins. In general, it appears that simple strength, intellective capacity, and the special senses are remarkably resistant, that endurance is much more sensitive, and that psychomotor functions like neuromuscular coordination and speed occupy an intermediate position (A. Keys *et al.*, *op. cit.* (*1945*); J. Brozek, H. Guetzkow, O. Mickelsen, and A. Keys, *J. Applied Psychol.* **30,** *359* (*1946*)). But the most important point is that in all acceptable experiments so far re-

ported, changes in fitness have been preceded by one or more of the following: anorexia, personality changes, signs of polyneuritis, oral or cutaneous alterations, increased pyruvic acid in the blood (M. C. Hulse *et al.*, *Ann. Int. Med.* **21,** *440* (*1944*); A. Keys *et al.*, *op. cit.* (*1945*); J. Brozek, H. Guetzkow, and A. Keys, *Psychosom. Med.* **8,** *98* (*1946*)).

The general conclusion from present evidence is that vitamin supercharging confers no benefit in terms of fitness and that fitness is not peculiarly sensitive to vitamin deficiencies. To be sure this evidence is based on relatively short-term experiments and while it is easy to suggest that much more research is needed, and the experimental period should be of long-term, it is only fair to point out the extreme difficulties in the way of really useful future research. Experiments extending for years instead of weeks or months are needed; the almost insuperable requirements for control are obvious. Better methods for evaluating fitness are needed, but these provide formidable problems in themselves. Not only are better measures of fitness required, but it must be pointed out again that this term does not include many other aspects of health such as maternal and infant health, longevity, and disease resistance, in which clearly demonstrable relations to improved nutrition have been shown.

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PRESENT KNOWLEDGE OF METHYL GROUPS IN NUTRITION

The concept of the methyl group as a dietary essential is unique in nutritional science in view of the simplicity of this chemical structure compared with the complexity and marked specificity of vitamins and other indispensable nutrients. The occurrence of demonstrable methyl deficiency on certain otherwise adequate diets has far reaching implications because of the biochemical importance of the principal sources of methyl and because of the fundamental role in metabolism of transmethylation, the transfer of a methyl group from one metabolite to another (*Nutrition Reviews* **2,** *358* (*1944*); **4,** *163* (*1946*)). A methyl group has but one form and whether a methyl-containing compound serves as a methyl donor depends upon the structure of the whole molecule rather than upon the methyl itself. The methyl is attached to nitrogen or sulfur in the known donors, the chief of which appears to be choline, methionine, and betaine. The terms "utilizable" and "labile" are commonly applied to methyls which participate in transmethylation. "Labile methyl" adequately expresses the idea of mobility but fails to describe accurately the methyl of creatine, for instance, which loses its lability following its incorporation into the creatine molecule. Demethylation does not necessarily give rise to utilizable methyl. The methyl of sarcosine may be removed in the body (L. D. Abbott and H. B. Lewis, *J. Biol. Chem.* **131,** *479* (*1939*)) but it is not available for transmethylation (V. du Vigneaud, J. P. Chandler, A. W. Moyer, and D. M. Keppel, *Ibid.* **131,** *57* (*1939*)). Transmethylation varies markedly according to species and individual methyl donors. In general, labile methyl is recognized in laboratory studies by one or more of the following characteristic functions: lipotropic action, the prevention of the accumulation of fat in the liver (*Nutrition Reviews* **1,** *168* (*1943*); **2,** *302* (*1944*); **3,** *143* (*1945*); **4,** *246*, *349* (*1946*)); promotion of growth in the presence of homocystine and in the absence of methionine (*Ibid.* **2,** *358* (*1944*)); prevention of renal hemorrhagic degeneration and related pathology (*Ibid.* **1,** *156* (*1943*); **2,** *207* (*1944*); **4,** *345* (*1946*)); prevention of perosis or "slipped tendon disease" in chicks (*Ibid.*

2, *50 (1944)*); and, appearance in tissues or body fluids of methyl compounds containing isotopic hydrogen previously administered in the form of a labeled methyl donor (*Ibid.* **4,** *306 (1946)*).

The chief sources of methyl in natural foodstuffs are choline in lecithin, methionine in protein, and, to a lesser extent, betaine. Evidence for the presence in food of an additional choline precursor, possible dimethylaminoethanol, has been obtained (H. L. Lucas, L. C. Norris, and G. F. Heuser, *Proc. Soc. Exp. Biol. Med.* **63,** *32 (1946)*). Choline and methionine are highly important metabolites wholly apart from their activity as methyl donors. Choline is a component of lecithin, sphingomyelin, and acetylcholine. It may be synthesized in the rat from a methyl donor and a carbon-nitrogen moiety which present evidence indicates is ethanolamine (*Nutrition Reviews* **2,** *358 (1944)*). The limiting factor controlling its production is believed to be the supply of the methyl donor, methionine, except in the chick (T. H. Jukes, *Poultry Sci.* **20,** *251 (1941)*). Methionine is an indispensable amino acid required for the synthesis of cystine and of tissue proteins (*Nutrition Reviews* **3,** *182 (1945)*). Whether a single molecule of methionine can yield both sulfur and methyl is an interesting but unsolved problem (D. J. Mulford and W. H. Griffith, *J. Nutrition* **23,** *91 (1942)*; F. Binkley and V. du Vigneaud, *J. Biol. Chem.* **144,** *507 (1942)*). The demethylation of methionine produces homocysteine which can be converted into methionine in the presence of a methyl donor (J. P. Chandler and V. du Vigneaud, *Ibid.* **135,** *223 (1940)*). The methylation of homocysteine may be an important method of conserving the supply of methionine but it is not a significant source of extra methionine because neither homocysteine nor homocystine occurs in natural foodstuffs. Methyl donors are used in the synthesis of creatine (*Nutrition Reviews* **2,** *358 (1944)*), anserine (J. R. Schenck *et al.*, *J. Biol. Chem.* **149,** *355 (1943)*), N′-methylnicotinamide (*Nutrition Reviews* **2,** *234 (1944)*), and possibly in the formation of epinephrine, ergothioneine, and the methylated selenium detoxication product. The administration of guanidoacetic acid (D. Stetten and G. F. Grail, *J. Biol. Chem.* **144,** *175 (1942)*) and of nicotinamide (*Nutrition Reviews* **2,** *234 (1944)*; **3,** *263 (1945)*) depletes the supply of methyl in the body. Transmethylation is not reversible with creatine (*Ibid.* **2,** *358 (1944)*) or N′-methylnicotinamide (*Ibid.* **4,** *345 (1946)*). Which methylations have priority, if the methyl supply is inadequate, is not known. The occurrence of the fatty liver as one of the first manifestations of methyl deficiency and the failure to demonstrate a similar disturbance of creatine formation (*Ibid.* **3,** *124 (1945)*) suggest that the utilization of methyl donors in the synthesis of creatine has priority over the formation of lecithin for transport of fatty acids away from the liver.

A dietary lack of methyl may result in impairment of one or more of the specific metabolic reactions which utilize choline and methionine as intact molecules and impairment of additional methylation mechanisms which depend upon an adequate methyl supply. The rate of turnover of choline phospholipids is affected by the availability of choline and this undoubtedly is the basis of the lipotropic action of choline and of methyl donors that favor its synthesis (*Nutrition Reviews* **2,** *275 (1944)*; **4,** *276 (1946)*). Whether the tissue pathology observed in choline deficiency is due to abnormal transport of fatty acids or to the failure of choline phospholipids to function normally as structural constituents of tissues is not known. Choline is effective in the prevention of the unexplained toxicity of ethyl laurate (*Ibid.* **3,** *239 (1945)*). The observation that certain compounds are lipotropic without being methyl donors is of great interest. Arsenocholine (*Ibid.* **2,** *358 (1944)*) and triethylcholine (C. S. McArthur, *Science* **104,** *222 (1946)*) are in this category and both have been shown to be incorporated into phospho-

lipid molecules in place of choline. A similar explanation of the lipotropic effect of inositol is an attractive but unverified hypothesis. The role of lipotropic factors such as inositol (*Nutrition Reviews* **4,** *163* (*1946*)) and lipocaic (D. E. Clark, M. L. Eilert, and L. R. Dragstedt, *Am. J. Physiol.* **144,** *620* (*1945*); *Nutrition Reviews* **4,** *187* (*1946*)) remains obscure. Little is known of a direct relation of choline to acetylcholine although it seems reasonable that the concentration and rate of turnover of acetylcholine should be dependent upon the methyl supply (D.Y. Solandt and C. H. Best, *Nature* **144,** *376* (*1939*)). Presumably, the use of choline in the synthesis of needed acetylcholine would have high priority in the body.

Choline is required for lactation in the rat (B. Sure, *J. Nutrition* **19,** *71* (*1940*)) and in the hamster (*Nutrition Reviews* **2,** *361* (*1944*)). Dogs are particularly susceptible to choline deficiency (*Ibid.* **2,** *298*, *314* (*1944*)); **3,** *124*, *261* (*1945*); **4,** *244* (*1946*)). Choline is one of several factors protecting against the development of lesions in the forestomach of rats (G. R. Sharpless and M. Sabol, *J. Nutrition* **25,** *113* (*1943*)). It is necessary for egg laying in hens and for the growth of chicks and young turkeys (*Nutrition Reviews* **2,** *358* (*1944*)). It is partially effective, at least, in the prevention of the deposition of cholesterol in the liver (*Ibid.* **4,** *349* (*1946*)). Choline inhibits the formation of ceroid pigment in the liver (*Ibid.* **4,** *137* (*1946*)) and methyl metabolism may be of importance in the carcinogenic action of dimethylaminoazobenzene (H. P. Jacobi and C. A. Baumann, *Cancer Research* **2,** *175* (*1942*)). Extremely significant is the report of the spontaneous development of neoplasms in rats maintained for long periods on a low choline diet (D. H. Copeland and W. D. Salmon, *Am. J. Path.* **22,** *1059* (*1946*)).

There have been relatively few reports of injurious effects following the administration of choline. Feeding excessive amounts has been associated with "bronzed pigmentation" in rats (*Nutrition Reviews* **4,** *49* (*1946*)). A possible connection between dietary choline and tissue acetylcholine is afforded by the observation of a surprising relationship between acetylcholine, cholinesterase, and the formation of red cells. Feeding of choline (*Ibid.* **4,** *83* (*1946*); **5,** *148* (*1947*)) and injection of acetylcholine cause an anemia which responds to folic acid or liver extract (J. E. Davis, *Am. J. Physiol.* **147,** *404* (*1946*)). This investigator has reported that the acetylcholine of serum is decreased in patients with pernicious anemia in whom therapeutic remission was induced by folic acid (J. E. Davis, *Science* **104,** *605* (*1946*)). The effect of folic acid appeared to be related to a greater cholinesterase activity in serum.

Choline is required for the growth of pneumococci (L. Rane and Y. Subbarow, *J. Bact.* **40,** *695* (*1940*)) and a mutant strain, Neurospora crassa cholineless, has been found which is useful in the determination of choline (N. H. Horowitz and G. W. Beadle, *J. Biol. Chem.* **150,** *325* (*1943*)). There is evidence of methyl synthesis by intestinal microorganisms (*Nutrition Reviews* **4,** *37*, *326* (*1946*); **5,** *13* (*1947*)).

The indispensability of methionine as an amino acid, its importance as a source of methyl, and the possibility that choline and other methyl donors may "spare" methionine, either in its role as a methyl donor or as an amino acid, emphasize the importance of this sulfur compound in methyl metabolism. The fact that it also serves as a source of cystine sulfur introduces the complicating factor of the sparing effect of cystine supplements in diets low in cystine and methionine. Methionine favors recovery after poisoning by arsenicals, chloroform, and carbon tetrachloride (*Nutrition Reviews* **2,** *292*, *340* (*1944*), **3,** *325* (*1945*)) and burns (*Ibid.* **3,** *223* (*1945*)). Choline and methionine together reduce the mortality of rats poisoned with ethylene dichloride (L. A. Heppel *et al.*, *J. Ind. Hyg. Toxicol.* **27,** *15* (*1945*)). Any specific effect of methionine is important in methyl metabolism because its use as an

amino acid decreases the available methyl supply. In this connection, the demonstration of an "antimethionine" effect by certain compounds is suggestive (*Nutrition Reviews* **4,** *70 (1946)*).

A direct relation between methyl, choline or methionine metabolism, and liver pathology in man and in animals is plausible because of the potentially injurious effect of chronic fatty infiltration and because of the apparent dependence of the liver upon an adequate supply of amino acids, particularly sulfur amino acids. Attempts to demonstrate such a relationship have not been uniformly successful (*Nutrition Reviews* **1,** *88 (1943)*; **2,** *292, 314, 340 (1944)*; **3,** *122, 158, 325 (1945)*; **4,** *27, 127, 180 (1946)*). However, the reality of hepatic degeneration in animals on experimental diets deficient in protein and in other nutrients is unquestioned. It is not surprising, perhaps, that clearcut therapeutic results are difficult to obtain after the appearance of severe hepatic pathology. Preventive dietary studies, although obviously difficult, may supply the answer to the vexing problem of cirrhosis and other liver disease in man.

Dietary sources of methyl should include both choline and methionine, inasmuch as these are not completely interchangeable as sources of methyl in all species. Furthermore, dependence upon methionine as a source of methyl is impractical because it involves wastage of amino acids. A casein level of 30 per cent is necessary to provide the young rat with sufficient methionine for methylations, including choline synthesis, whereas approximately two thirds of this quantity of casein is adequate if dietary choline is supplied (D. J. Mulford and W. H. Griffith, *J. Nutrition* **23,** *91 (1942)*). Among natural foodstuffs whole egg protein is the richest source of methionine and total sulfur. Compared with whole egg protein as a standard, methionine is the limiting amino acid in lactalbumin and in proteins of human milk, peanut, soybean, pea, and corn germ. On the same basis, methionine and cystine are the limiting amino acids in casein and in the protein of cow's milk, beef muscle, beef kidney, and yeast and are in second place as limiting amino acids in gelatin and in the proteins of beef heart, beef liver, alfalfa, rolled oats, and wheat germ (H. H. Mitchell and R. J. Block, *J. Biol. Chem.* **163,** *599 (1946)*). Dietary protein which is low in total sulfur may increase the hazard of methyl deficiency as a result of the utilization of methionine as a source of cystine sulfur rather than as a methyl donor. Choline occurs chiefly in the form of phospholipids in natural foodstuffs and its concentration varies according to the lecithin content (*Nutrition Reviews* **1,** *312 (1943)*).

Studies on the nutritional aspects of methyl have contributed enormously to the fuller understanding of the term "optimum diet." These investigations have answered some questions and have introduced more problems which remain to be solved. Abnormal metabolism, due to disease or other cause, may increase the demand for methyl, for choline, or for methionine so that a borderline dietary supply may become insufficient and methyl deficiency may be superimposed on the original pathologic condition. The use of low protein diets as an experimental means of obtaining low methionine diets has been fruitful but yields results which are difficult to interpret because of the modification of specific deficiencies in the presence of multiple deficiencies. Particularly desirable, therefore, is the determination of the effect of methyl deficiency uncomplicated by deficiencies in protein and in other nutrients.

ROLE OF THE LIVER IN PLASMA PHOSPHOLIPID METABOLISM

Although the reaction involving incorporation of inorganic phosphate into phospholipids is not restricted to any one tissue, M. C. Fishler, C. Entenman, M. L. Montgomery, and I. L. Chaikoff (*J. Biol. Chem.* **150,** *47 (1943)*) showed that the phospholipids contained in the plasma are synthesized mainly by the liver. The function of the liver in the formation of plasma phospholipids was demonstrated by comparing the rates at which labeled inorganic phosphate introduced into the blood stream was converted to labeled plasma phospholipids by the normal and liverless dog. Negligible amounts of labeled phospholipids were recovered in the plasma of the latter as late as six hours after excision of the liver.

C. Entenman, I. L. Chaikoff, and D. B. Zilversmit (*J. Biol. Chem.* **166,** *15 (1946)*) have published an account of the removal of plasma phospholipid. After intravenous injection of labeled phospholipid, the specific activity of plasma phospholipid phosphorus of dogs was measured. This specific activity did not decrease significantly after the liver had been excluded from the circulation. It would appear, therefore, that not only is the liver responsible for the synthesis, but also principally for the utilization of plasma phospholipid.

This interpretation raises the question as to what function the plasma phospholipid serves in the animal body. The apparent importance of the liver in contrast to the total mass of extrahepatic tissue in the removal of plasma phospholipid would seem to be in contradiction to the traditional view that the main function of plasma phospholipid is to transport fatty acids from the liver to other tissues. The failure of fat deposition in extrahepatic tissues in any conditions adversely affecting phospholipid synthesis is strong support of this widely held view. It must be considered that the incorporation or loss of labeled phosphorus from phospholipid does not necessarily prove complete synthesis or breakdown of phospholipid, but strictly only that the phosphorus of phospholipids is exchanged with inorganic phosphate. Too little is known of the changes which occur in phospholipids in their extrahepatic metabolism. Finally, it is held by many, with some justification, that removal of the liver may produce profound alterations in the normal metabolism of the other tissues. The apparent conclusion reached by the isotope experiments should be tempered with these considerations.

INTELLECTUAL FUNCTIONS WITH RESTRICTED INTAKES OF B-COMPLEX VITAMINS

The Association for Research in Mental and Nervous Diseases utilized an entire annual meeting for a consideration of the effects of nutritional deficiencies upon behavior (*Res. Pub. Assn. Nerv. Ment. Dis.* **22** *(1943)*). Particular attention was paid to the vitamins of the B-complex because of their known importance in the metabolism of nerve tissues. These papers showed that the experimental evaluation of intellectual and personality changes is less advanced than biochemical research dealing with the effects of vitamin B restriction. Significant contributions have been made, however, by interdisciplinary nutritional research (J. Brozek and A. Keys, *Am. Scientist* **33**, *103 (1945)*) dealing with the application of standardized observational technics to human problems of sensory and motor performance, emotional adjustment, and intellectual functions.

H. Guetzkow and J. Brozek (*Am. J.*

Psychol. **59,** *358 (1946)*) have given a detailed report of the intellective performance of 8 men maintained on experimentally varied intakes of vitamins of the B-complex. The use of test technics standardized upon known populations provided meaningful measures of the subjects' behavior before, during, and after the experimental regimen. Some of the limitations of working with unavoidably small numbers of subjects were thus avoided. After a standardization period of forty-one days these men were studied during one hundred and sixty-one days of partial dietary restriction of thiamine, riboflavin, and niacin. The diet used provided approximately 3300 calories per day with an intake of 0.185 mg. of thiamine, 0.287 mg. of riboflavin, and 3.71 mg. of niacin per 1000 calories. Other components of the vitamin B-complex were probably reduced to a similar extent. During prolonged partial restriction the experimental unsupplemented group thus received about one third of the thiamine and riboflavin allowances recommended by the National Research Council, and two thirds of the amount of niacin. During a subsequent twenty-three day period, 4 men were maintained on a diet practically free of vitamins in the B-complex.

During the period of partial restriction the subjects showed no intellective deterioration as measured by the two tests of general intelligence (American Council on Education and the Ohio State University tests) and the Porteus mazes. There was no evidence that this prolonged restriction affected learning ability as measured by the above tests as well as other tests requiring adjustment to new and varied tasks, test of spatial relations, reasoning, and verbal fluency.

At the end of partial restriction two widely used clinical tests (Shipley-Hartford Retreat Scale and Hunt-Minnesota Test for Organic Brain Damage) gave scores indicating no pathology. It is to be noted that during the same period biochemical studies (A. Keys *et al., Am. J. Physiol.* **144,** *5 (1945)*) showed an increase in blood pyruvic acid which suggested that the vitamin B-complex intake was not sufficient to maintain normal metabolism.

A very limited deterioration in intellective functions was apparent in the men subjected to an acute twenty-three day deprivation. There was a downward change in the general performance level which was most apparent in tests requiring speed. While intellective capacities during acute deficiency remained essentially intact there was a marked decrease in spontaneous activities such as reading, writing letters, study, or conversation. Apparently marked emotional and personality changes did occur during this time (J. Brozek, H. Guetzkow, and A. Keys, *Psychosom. Med.* **8,** *98 (1946)*). There was a pronounced rise in psychoneurotic scores on the Minnesota Multiphasic Personality Inventory. These personality changes were apparent only during acute deficiency and indicated depression and hypochondriases. It is interesting that these changes appeared early in the acute deficiency, just after the development of anorexia. The metabolic, neurologic, and cardiovascular changes appeared later. A rapid recovery was noted in the impaired subjects during a ten-day supplementation with thiamine alone.

It seems that these results should not be overgeneralized. The authors point out that they apply to normal young men. The subjects used were highly motivated volunteers (conscientious objectors). There is some possibility that such subjects might be able to compensate for the effect of personality changes which would be vastly accentuated in mental patients, seniles, or young children. This might explain the disparity of these recent results with the earlier clinical reports of W. H. Sebrell (*Res. Pub. Assn. Nerv. Ment. Dis.* **22,** *113 (1943)*) and T. D. Spies, J. Bradley, M. Rosenbaum, and J. R. Knott (*Ibid.* **22,** *122 (1943)*) in which marked intellectual changes were noted in patients having vitamin B-complex deficiencies. Disorientation and memory losses were observed in these sub-

jects but the authors remark that such changes may be secondary to emotional disturbances. No measures of intellectual capacity were employed in these studies.

Earlier clinical studies of vitamin B-complex deficiency in older women by H. E. O'Shea and K. O. Elsom (*Am. J. Med. Sci.* **203,** *388 (1942)*) also noted decrease in those tests emphasizing speed while no changes were found in psychometric tests of mental ability. G. L. Wadsworth *et al.* (*Am. J. Psychiat.* **99,** *807 (1943)*) found no decrease in the scores of senile mental patients on a battery of intelligence and memory tests in which speed was not stressed.

The importance of age as a factor in the effects of vitamin B-complex deficiencies upon performance has been established in animal studies reviewed by W. C. Biel and D. D. Wickens (*J. Comp. Psychol.* **32,** *329 (1941)*). This factor is probably of extreme importance in human studies (R. F. Harrell, *Teachers Coll. Contr. Educ., Columbia Univ.* **877,** *1 (1943)*) on mentally retarded children given B-complex supplements. Here, however, Harrell found no increase in performance capacity, only a decrease in fatigability.

There is a necessity for considering the age, motivation, and physical condition of the subjects before a direct comparison of experimental studies may be made. There seems to be no clear evidence of impairment of intellectual capacity during experimentally induced deficiencies in the B-complex. There does seem to be a slight decrement in tests requiring speed or continued concentration. Such changes, however, are present during severe deficiency states and are accompanied by changes in mood (depression) and a lack of motivation for voluntary activities.

For a fuller understanding of the effects of nutritional deficiencies upon the varied aspects of human behavior, close cooperation is needed between research workers representing different fields of scientific specialization. The above study of Guetzkow and Brozek represents one aspect of such a program.

PYRIDOXAL PHOSPHATE AS A COENZYME IN TRYPTOPHANE FORMATION

Remarkably rapid progress has been made in the elucidation of the metabolic functions of pyridoxine (vitamin B_6). The work of several groups of investigators which culminated finally in the demonstration that pyridoxal phosphate functions as the coenzyme for a number of bacterial enzymes, catalyzing the decarboxylation of l-amino acids, has been reviewed by E. F. Gale (*Advances in Enzymology* **6,** *1 (1946)*; *Nutrition Reviews* **3,** *72 (1945)*). More recently, it has been clearly established that the same coenzyme is also essential for the activity of bacterial enzymes catalyzing transamination (*Ibid.* **4,** *232 (1946)*). The coenzyme is distributed widely in animal tissues, as well as in bacteria (E. F. Gale, *loc. cit.*) so that its function is obviously not limited to bacterial metabolism. Pyridoxal phosphate is now known to be essential for at least one decarboxylase of animal origin and there is strong evidence that it is also an essential component of the transaminases of animal tissues (*Nutrition Reviews* **4,** *232 (1946)*). Indeed, the earliest evidence for the latter function of pyridoxal phosphate was derived from studies of pyridoxine deficient animals.

Most recently, a third function for pyridoxal phosphate has been announced by the group at Cornell University which had been very active in the earlier elucidation of the role of this substance in both decarboxylation and transamination. W. W. Umbreit, W. A. Wood, and I. C. Gunsalus (*J. Biol. Chem.* **165,** *731 (1946)*) report that pyridoxal phosphate acts in the formation of tryptophane from indole and serine.

E. L. Tatum and D. Bonner (*Nutrition Reviews* **2,** *243 (1944)*) had previously demonstrated the biosynthesis of tryptophane from

indole and serine in the mold Neurospora. They believed that a direct reaction between indole and serine, possibly an intermolecular dehydration, took place:

indole + serine → tryptophane + water

Umbreit, Wood, and Gunsalus (*loc. cit.*) have now obtained from the frozen mycelium of a strain of Neurospora a cell-free extract which catalyzes the same reaction. When the mycelium was kept frozen for several weeks before extraction, the reaction between indole and serine did not take place to an appreciable extent. However, when pyridoxal phosphate was added the extract was reactivated and tryptophane was again formed.

A remarkable feature of this reaction in Neurospora extracts is that it appears to proceed quantitatively in the direction of tryptophane synthesis. According to Tatum and Bonner (*loc. cit.*) the well known formation of indole from tryptophane in E. coli is the result of a reversal of the above reaction, the equilibrium being shifted presumably in this case by oxidative removal of the serine as it is formed. It will be of considerable interest to determine whether pyridoxal phosphate is essential for the direct hydrolytic cleavage of tryptophane as well as for its synthesis.

The metabolism of tryptophane in animal tissues is known to be altered profoundly in pyridoxine deficiency, large amounts being excreted in the form of xanthurenic acid. When adequate amounts of pyridoxine are present in the diet, the metabolism of tryptophane appears to follow a different course, not involving xanthurenic acid as an intermediate (*Nutrition Reviews* **3**, *72 (1945)*). Umbreit, Wood, and Gunsalus have refrained from speculation on the relationship of their new finding to this problem. However, it seems safe to predict that pyridoxal phosphate will be found to play an essential role in the normal metabolism of tryptophane in animal tissues, and from the results just mentioned it appears possible that the particular reaction concerned may be the hydrolytic removal of the side-chain of tryptophane.

CARDIAC CHANGES IN ANEMIA

Nutritional deficiencies can result in development of nonspecific secondary lesions of primary importance. This generalization is illustrated by A. Hunter (*Quart J. Med.* **15**, *107 (1946)*) in an extensive review of the varied cardiac changes which are associated with anemia. Conditions such as cardiac murmur, enlargement of the heart, fatty changes in the cardiac muscle, and electrocardiographic abnormalities were noted.

Cardiac enlargement has often been attributed to myocardial anoxemia accompanying the anemic state. More recent studies reviewed by Hunter attribute the cardiac enlargement in part to gross disturbances of the circulation in anemia, such as a raised cardiac output and increased venous pressure. Note should be taken that the heart size often returns to normal after treatment of the anemia. Cardiac pain, indistinguishable from that occurring as angina pectoris, has not infrequently been noted as accompanying anemia. In some cases this pain has been closely associated with other cardiac signs such as tachycardia, murmurs, and enlargement, and it has sometimes been demonstrated that no diseases of the coronary arteries existed to account for the pain.

Hunter reports a summary of his experience with 34 patients, 27 of whom had hypochromic anemia with or without chronic blood loss, 5 of whom had Addisonian anemia, and 2 with refractory anemias. The initial hemoglobin values of his patients ranged from 22 to 55 per cent (standard not stated). The duration of the anemia had been from six weeks to twenty years. All patients with known or reputed valvular disease or hyper-

tension were excluded from the study. The individuals were observed during the course of treatment until their hemoglobin levels had returned to 90 to 100 per cent, except when cardiac findings were negative and then observation was sometimes discontinued when the hemoglobin levels had attained 80 per cent. The period of observation was usually several months but sometimes extended over a year.

Of the symptoms which were most suggestive of cardiac disease, dyspnea was the most common. It occurred in all but 2 patients. Treatment of the anemia always relieved this symptom. Orthopnea was never present. Palpitation was noted in 18 of the 34 patients. Cardiac pain similar to that seen in angina pectoris was present in 8 of the 34 cases or approximately 25 per cent of the series. Complete abolition of the pain occurred in every case after therapy for the anemia. It is of considerable interest that hypertension was never present at the first examination, but was manifest in 2 of the older patients after the anemia had been corrected. This dissociation of the anginal type of pain with hypertension led the author to suggest that any woman under 40 years of age without hypertension and complaining of cardiac pain should be considered as possibly presenting an unrecognized anemia.

Among the physical signs frequently noted by this investigator were changes in the tongue and fingernails (see *Nutrition Reviews* **4,** *263 (1946)*). Cardiac murmurs were noted in all but 4 of the patients prior to therapy. Sixteen of the patients exhibited murmurs after treatment for the anemia. Prior to treatment 12 of the patients exhibited definite cardiac enlargement while 6 had a doubtful cardiac enlargement. Only 3 of the patients exhibited definite cardiac enlargement after the anemia was corrected and 4 remained doubtful. This reduction in heart size is illustrated by the reproduction of two x-rays of patients with hypochromic anemia taken before and after treatment. The authors do not feel that the cardiac enlargement is the cause of the so-called hemic murmurs. The electrocardiographic changes were varied in character and did not always revert to normal upon successful treatment of the anemia.

The author points out that inasmuch as the enlargement of the heart which he observed often disappeared rapidly while the patient was under treatment and before the hemoglobin had returned to normal, it seems reasonable that myocardial anoxemia does not entirely explain these changes. Instead, he attributes the enlargement to a dynamic disorder of the circulation.

It would seem pertinent to raise the question whether these evidences of myocardial damage might not be attributable, at least in part, to localized impairment of the metabolism of the myocardium itself. This hypothesis would seem especially worthy of investigation inasmuch as hypochromic anemia is due to iron deficiency and the iron-containing respiratory pigments play a considerable role in the metabolism of muscle. Also, the metabolic defect which occurs in Addisonian pernicious anemia results in a deficiency of a substance which may have rather widespread importance in myocardial metabolism. Irrespective of the causative mechanism, it is worthy of emphasis that here are two additional deficiencies which result in cardiac damage—a type of deficiency manifestation which students of nutrition often mistakenly associate specifically with thiamine deficiency.

DIETARY PROTEIN AS A SOURCE OF PROTOPORPHYRIN

Protoporphyrin is the predominant porphyrin which occurs in the feces of the rat (M. O. Schultze, *J. Biol. Chem.* **142,** *89 (1942)*) while only a trace is eliminated in the urine, even after the parenteral administration of protoporphyrin. There is relatively little protoporphyrin in the body except in the Harderian glands (R. H. McCoy, *Abstracts*

of Pittsburgh Meeting (106th), American Chemical Society, page 20 (1943)), the intestinal contents (McCoy, *loc. cit.*) the erythrocytes, especially the reticulocytes (M. Grinstein and C. J. Watson, *J. Biol. Chem.* **147,** *675 (1943)*), and in the white matter of the central nervous system (H. Klüver, *Science* **99,** *482 (1944)*).

It appears that the fecal protoporphyrin is chiefly protoporphyrin formed in excess of the requirement for hemoglobin synthesis, and excreted by way of the Harderian glands, since it has been shown by McCoy (*loc. cit.*) that removal of these structures reduces fecal protoporphyrin almost to zero in the rat. The amount of protoporphyrin synthesized by the microorganisms of the intestinal tract has also been shown to be inconsiderable (McCoy, *loc. cit.*; A. Jakob, *Klin. Wchnschr.* **18,** *1024 (1939)*). In view of the above evidence, it may be assumed that protoporphyrin synthesis in the rat could be determined by measuring the amount excreted. Until recently, such a measurement would have been impossible, but Grinstein and Watson (*loc. cit.*) in 1943 described a method for estimating the protoporphyrin content of erythrocytes which appears suitable with modification for application to the determination of the fecal protoporphyrin. An attempt has therefore been made by J. M. Orten and J. M. Keller (*J. Biol. Chem.* **165,** *163 (1946)*) to study the excretion of fecal porphyrin in rats maintained on normal and low protein diets, to secure evidence that dietary protein serves as its precursor.

Male rats were given a diet of sucrose, corn dextrin, hydrogenated cottonseed oil, and salt mixture, supplemented with ryzamin B, liver extract, and haliver oil with viosterol to provide the vitamins. Twelve rats were maintained on a "normal" diet containing 22.5 per cent casein, and 12 litter mates were kept on a diet containing only 3.5 per cent casein, but a correspondingly greater quantity of dextrin. Weight and food consumption were recorded weekly. The feces were collected on absorbent paper as completely as possible and duplicate determinations of the protoporphyrin were made biweekly for each rat, by a modification of the method of Grinstein and Watson (*loc. cit.*). Hemoglobin determinations for each rat were made at regular intervals.

The results showed that the fecal content of protoporphyrin of the rats on the "normal" diet averaged 42 micrograms per gram of dried feces after four weeks on the experiment, and during the subsequent sixteen weeks rose to about 90 micrograms per gram. Expressed as micrograms of protoporphyrin per rat per day, the amount was 110 micrograms daily at four weeks, rising to more than 200 micrograms daily at the end of the period. In relation to the weight of protein ingested per day the protoporphyrin rose from 62 micrograms per gram of protein at the beginning to about 100 micrograms per gram of protein at the end of the experiment. For rats on the low protein diet the results were different. The amount of protoporphyrin per gram of dried feces was 29 micrograms after two weeks, rose slowly for four weeks, and then declined to 12 micrograms. Expressed as protoporphyrin per rat per day there was a slow continuous fall from 19 micrograms to 6 micrograms, while in terms of protein ingested the protoporphyrin showed a tendency to rise from 127 micrograms per gram after two weeks to 153 micrograms per gram after six weeks, but then declined to about 95 micrograms per gram at the end of the experiment.

It is thus evident that the control rats on a "normal" diet excreted more protoporphyrin per gram of feces or per day than did the animals on the low protein diet. This difference was evident within four weeks of starting, and became more noticeable as the experiment continued. The most instructive comparison was made on the basis of micrograms of protoporphyrin excreted per 100 g. of body weight per day; for the normal control rats the amount was almost constant throughout at approximately 50 micrograms per 100 g. per day, while for the rats on low

protein diet it declined steadily from 41 micrograms to 13 micrograms per 100 g. per day. This decrease was paralleled by the onset of anemia in the protein deficient rats. In the early part of the experiment the low protein rats showed a much greater utilization of the protein than the normal controls, but this difference vanished by the end of the experiment. As reasons for this the authors suggest a general failure in hematopoiesis and the decreased rate of growth, each making more protein available for porphyrin formation.

From these data it is therefore argued that protoporphyrin synthesis is a high priority reaction of the organism. There is little evidence of the specific amino acids which enter into the formation of protoporphyrin. D. Shemin and D. Rittenberg (*J. Biol. Chem.* **159,** *567 (1945)*; *Federation Proceedings* **5,** *153 (1946)*) have demonstrated that the nitrogen of glycine but not proline, glutamic acid, leucine, or ammonium citrate can be built into the protoporphyrin molecule. It may be noted too that K. Bloch and D. Rittenberg (*J. Biol. Chem.* **159,** *45 (1945)*) have also shown the utilization of acetic acid in the same synthesis. If glycine and acetic acid are the only precursors required for the synthesis of protoporphyrin, then the relations demonstrated by Orten and Keller indicate that an insufficient amount of nitrogen is provided for the synthesis on a protein deficient diet, although this is undoubtedly complicated by the relative needs for this dietary protein in the maintenance of tissues other than blood.

ASCORBIC ACID AND THE B-VITAMINS IN RELATION TO SEVERE INJURY

In the care of surgical patients all known vitamins are being used with increasing frequency and in increasing doses. Studies of ascorbic acid and of vitamin K in relation to surgical conditions have been reported in a number of publications but little has as yet been stated in regard to riboflavin, thiamine, or niacin. W. M. Govier and co-workers (see *Nutrition Reviews* **3,** *74, 89 (1945)*) reported that the mortality of normal dogs exposed to a standardized type of hemorrhagic shock was reduced when thiamine was given in large doses before or after bleeding. In addition, he found that dogs deficient in thiamine were particularly susceptible to death from hemorrhagic shock. G. Sayers, M. A. Sayers, T.-Y. Liang, and C. N. H. Long (*Endocrinology* **38,** *1 (1946)*) found that there is a sudden fall in the ascorbic acid content and a slower fall in the cholesterol content of the adrenal glands in hemorrhage and in hemorrhagic shock in rats and guinea pigs. W. A. Andreae, V. Schenker, and J. S. L. Browne (*Federation Proceedings* **5,** *3 (1946)*) studied the excretion of riboflavin in 23 cases of burns and injuries. Their subjects were given 5 mg. of crystalline riboflavin by mouth. Whereas only about one half of the ingested riboflavin was found by these investigators to be retained at this intake level in health, patients with acute injuries characteristically showed a marked retention during the initial three to five days after injury. This state was followed by a period of similar duration when there occurred an increased riboflavin loss as shown by the excretion rate. After about ten days following the injury the riboflavin balance returned to normal. This suggested that the vitamin retained during the first period was not utilized or destroyed but was stored in some way and subsequently released. There was a striking correlation between the retention of riboflavin and the observed retention of nitrogen during convalescence in these subjects.

S. M. Levenson *et al.* (*Ann. Surgery* **124,** *840 (1946)*) undertook to study the problem further. Their patients were observed in a metabolic ward under satisfactory conditions. Standard methods were used for the measurement of vitamin levels. Vitamin supplements were given to the subjects in capsules

containing 3 mg. of thiamine chloride, 3 mg. of riboflavin, 1.5 mg. of pyridoxine, 5 mg. of pantothenic acid, 25 mg. of nicotinamide, and 75 mg. of ascorbic acid in addition to tocopherols and vitamins A and D. Saturation tests were carried out by giving the test doses after the collection of a fasting specimen of blood and a fasting hour specimen of urine. The specimens of blood and of urine were then collected at intervals of one-half, one, two, three, and four hours. Two different test doses were used; one, the "high test" contained not less than 1 g. of ascorbic acid, 20 mg. of thiamine chloride, 12 mg. of riboflavin, and 450 mg. of nicotinamide; the "low test" contained 250 mg. of ascorbic acid, 5 mg. of thiamine, 3 mg. of riboflavin, and 50 mg. of nicotinamide.

Six patients with severe acute surgical conditions were studied. A well developed and well nourished healthy male with major injuries from bullet wounds but not in shock and with a good urinary output, showed zero plasma ascorbic acid on two occasions prior to operation. These levels rose slightly during recovery from operation. The first determination of thiamine output was low but the second and both determinations of riboflavin and of N′-methylnicotinamide were normal. In a patient severely injured while intoxicated the ascorbic acid concentration on three occasions during the first twelve hours was zero. At sixteen hours, without any ascorbic acid having been given the plasma level had risen to 0.3 mg. per 100 ml. At that time a high saturation test was performed. The plasma response to this was lower than is seen in normal individuals. The excretion was also low, being less than 50 mg. The next day this test was repeated. The plasma curve was almost identical but nearly 300 mg. were excreted. This was still below the usual excretion of a fully saturated individual. The fasting hour excretions of the three B-vitamins and ascorbic acid were at low levels immediately after injury and remained at these low levels for five days despite large doses given daily. Again, in a boy who had a severe injury but was in excellent muscular training and nutritional condition and who did not go into shock, the ascorbic acid concentration in the plasma remained relatively high from the beginning, being 0.6 mg. per 100 ml. of plasma on two occasions prior to any vitamin supplementation. He was given 1 g. of ascorbic acid, 30 mg. of thiamine, 12 mg. of riboflavin, and 450 mg. of nicotinamide daily. In addition, high saturation tests were performed. Nevertheless, the plasma levels of ascorbic acid in the first two tests were low and the four hour urine excretions of ascorbic acid, thiamine, and riboflavin during saturation tests were low during the first two tests but became normal when the tests were repeated on the seventh day. The excretion of N′-methylnicotinamide was normal on all occasions. In another instance which concerned a relatively old but muscular laborer with extremely severe injuries and a long period of shock, the blood level of ascorbic acid and the urinary excretion of all four of the vitamins measured remained extremely low in comparison with the enormous intakes until the eighth day when they all increased to excretion levels which were normal for his intake. Similar observations were made in 2 other subjects.

Thus in all 6 patients with severe injuries, hemorrhage, or infection, abnormalities in the metabolism of ascorbic acid, thiamine, riboflavin, and nicotinamide appeared to exist although they varied in extent and duration. There was a low concentration of ascorbic acid in the plasma, fasting or after saturation tests, and low excretion of any of the four vitamins under the same two conditions. The abnormalities were most marked when the patients were most sick; that is, when the metabolic disturbance of the carbohydrates, proteins, and adrenocortical hormone were also most marked.

An explanation for these observations is not too obvious. The investigators point out that all of these subjects had no recognizable signs or symptoms of deficiencies of any of the vitamins studied. Although some of

the subjects might have been less than fully saturated, it is pointed out that even in scurvy the plasma levels, fasting and following therapy, and the excretion following therapy rise much more rapidly after treatment than was observed in these subjects. Since even the patients given the largest doses of vitamins showed slight excretion during the acute phases of their illnesses, to be followed by very active excretions as soon as their metabolic disturbances were corrected, it would not appear that inadequate dosage of vitamins administered was a factor. Since most of the vitamins were given intravenously, failure of absorption was not a factor. It has been shown that sweating cannot cause important losses of these vitamins and losses of vitamins into exudates could not have been factors since only one of the 6 patients had any significant exudation. No data in the study could be used to determine whether some alteration in storage of vitamins had occurred, as is suggested by the observations of Andreae and his associates (*loc. cit.*). There is no information, also, as to whether the vitamins given might have been destroyed without performing any useful function. It is plausible to assume, even though there is no proof, that under the conditions of stress which developed in these subjects there was an increased requirement for vitamins and increased utilization.

ANIMAL ASSAY OF VITAMIN B_6 WITH SYNTHETIC RATIONS

A number of rations were used for the bioassay of vitamin B_6 before the newer members of the vitamin B-complex were available in synthetic form. Now that such factors as synthetic biotin and folic acid may be obtained, P. S. Sarma, E. E. Snell, and C. A. Elvehjem (*J. Biol. Chem.* **165,** *55 (1946)*) have investigated the possibilities of using synthetic rations which would permit minimum growth of rats on the deficient basal diet and maximum growth when optimum amounts of vitamin B_6 were added.

In the attempts to devise the most suitable basal ration, a start was made with one containing 75 per cent carbohydrate, 18 per cent protein, 3 per cent corn oil, and 4 per cent of salt mixture, together with optimum amounts of thiamine, riboflavin, calcium pantothenate, choline chloride, 2-methyl-1,4-naphthoquinone, inositol, niacin, and biotin. Male weanling rats of the Sprague-Dawley strain at 21 days, weighing 35 to 40 g., were used in the experiments. Different carbohydrates and proteins were tried, with the object of finding the combination which would permit minimum growth gain in the absence of vitamin B_6. Eighteen per cent casein was found to give more growth than 18 per cent fibrin whatever carbohydrate was used. When the carbohydrate was sucrose, the diet containing fibrin produced a growth of only 5 g. per week as against 9 g. when casein was used. This difference was greater than could be accounted for by the fact that the casein contributed 4.7 micrograms of vitamin B_6 per 100 g. of ration while the fibrin contributed only 1.0 micrograms as measured by the yeast growth method of L. Atkin, A. S. Schultz, W. L. Williams, and C. N. Frey (*Ind. Eng. Chem. (Anal. Ed.)* **15,** *141 (1943)*). When glucose was used the results were similar to those with sucrose, but when dextrin was used a considerable increase in growth was observed, even though the dextrin was shown by yeast growth assay to contain only 0.04 microgram per gram of vitamin B_6. This was due presumably to the well-known property of dextrin to stimulate intestinal synthesis by microorganisms; a possibility which was tested experimentally by adding sulfathalidine and sulfathalidine plus excess pyridoxine to the diet. The results at no point contradicted the explanation suggested.

This effect of dextrin was also confirmed by analysis of the urine of rats maintained on sucrose and dextrin rations. S. Lepkovsky

and E. Nielsen (*J. Biol. Chem.* **144,** *135* (*1942*)) showed, and E. C. Miller and C. A. Baumann (*Ibid.* **157,** *551* (*1945*)) confirmed that the amount of xanthurenic acid excreted in the urine by rats kept on a high tryptophane diet depends on the amount of vitamin B_6 in the diet. J. W. Huff and W. A. Perlzweig (*Ibid.* **155,** *345* (*1944*)) have established that the chief metabolic product of vitamin B_6 occurring in human urine is 4-pyridoxic acid. When rats on the fibrin and sucrose diet were compared with others on the fibrin and dextrin diet, it was found that those receiving dextrin excreted 50 per cent more 4-pyridoxic acid in twenty-four hours and one-third as much xanthurenic acid per gram of food ingested. As the two diets were equally deficient in vitamin B_6, it must be concluded that the intestinal flora is stimulated by dextrin to produce the extra vitamin B_6 required for these effects.

It was evident from the above results that the carbohydrate to be used for the basal ration should not be dextrin, and that fibrin was the better protein tested. The final composition adopted for the basal ration was: sucrose 75 g., fibrin 18 g., salts 4 g., corn oil 3 g., thiamine 0.2 mg., riboflavin 0.3 mg., niacin 2.5 mg., calcium pantothenate 2 mg., 2-methyl-1,4-naphthoquinone 1 mg., inositol 10 mg., choline chloride 100 mg., and biotin 0.01 mg., with the addition of haliver oil and tocopherol to supply the fat soluble vitamins.

This basal ration was used to deplete the rats for two weeks, after which time groups of rats were given different amounts of vitamin B_6 as pyridoxine hydrochloride. The curve of response of the rats was found to be approximately linear up to 75 micrograms of pyridoxine hydrochloride per 100 g. of diet (27 g. gain in weight per week), although twice this amount was required to give the maximum growth rate of just over 30 g. per week. The authors also used this diet to clear up the apparent contradictions in the work of various investigators on the relative vitamin B_6 activities of pyridoxine, pyridoxamine, and pyridoxal. Pyridoxine hydrochloride, pyridoxamine dihydrochloride, and pyridoxal hydrochloride were administered by mixing with the basal ration, by feeding them separately from a medicine dropper, and by intraperitoneal injection. Pyridoxamine and pyridoxal in equimolar amounts produced less weight gain than pyridoxine when mixed with the ration, although when fed by dropper or injected the three forms of vitamin B_6 produced the same growth response within the limits of experimental error. The same result was obtained with chicks on an appropriate basal ration.

The new ration is particularly useful in the assay of vitamin B_6 when the supplements can be fed by dropper or injected, since the different forms of vitamin B_6 are equally active in equimolar quantities. Although the latter is also true of the yeast growth assay of E. E. Snell (*J. Biol. Chem.* **157,** *491* (*1945*)), the animal assay method will probably remain of considerable value for determination of the vitamin B_6 activity of foods.

NUTRITIVE VALUE OF LEGUME PROTEIN AND METHIONINE SUPPLEMENTATION

Plant proteins are believed to be inferior to animal protein in nutritive value, but the difference may depend only on one or two of the essential amino acids. Lysine is believed to be a governing factor in the biologic value of certain seed proteins (*Nutrition Reviews* **1,** *178* (*1943*)), and addition of methionine to the diet enhances the biologic value of peas (E. Woods, W. M. Beeson, and D. W. Bolin, *J. Nutrition* **26,** *327* (*1943*)) and of soybean protein (H. J. Almquist, E. Mecchi, F. H. Kratzer, and C. R. Grau, *J. Nutrition* **24,** *385* (*1942*)). Some attention has been given to the protein

value of legumes commonly consumed by man (*Nutrition Reviews* **2,** *239* (*1944*)), but insufficient to determine their relative value, especially with regard to variety, and the extent to which their value might be enhanced by methionine. These latter points were the objectives of a study by W. C. Russell, M. W. Taylor, T. G. Mehrhof, and R. R. Hirsch (*J. Nutrition* **32,** *313* (*1946*)).

In this study the growth response of young white rats was used as a measure of biologic value. Four varieties of lima beans, five of snap beans, and nine of peas were used, in addition to the Bansei soybean and dried whole beef as reference protein sources. Chick peas, earlier observed to have a high nutritive value, were also included. All were fed at a 10 per cent protein level, the rest of the diet being synthetic and supplying adequate amounts of other nutrients. The legume seeds were washed, soaked, cooked, dried at 50° to 60°C., and pulverized before addition to the diet. Additional growth period studies were made in which 0.1 and 0.6 per cent methionine was included in the diet. An observation period of ten days appeared to reveal as much as longer periods, and 6 rats (3 male and 3 female) were used in each assay. A chemical assay for methionine was also applied to most of the products.

As a reference, dried whole beef provided an average of 3 g. daily weight gain. In contrast the greatest gain for any of the pea, snap bean, or lima bean varieties was 0.4 g. Soybean gave a 1.6 g., and chick peas a 1.2 g. gain. Varietal differences were considered insignificant, and the protein value of any but soybean and chick pea obviously low. When 0.1 per cent methionine was included in the diet, the weight gains on the legume diets increased greatly. In one variety of lima bean this gain was the same (3.0 g.) as for dried whole beef, and with most of the other legume varieties reached 50 to 65 per cent of this value. The value of soybean and chick pea was doubled and therefore essentially reached the value of unsupplemented dried whole beef (this was itself increased from 3.0 to 3.7 g. by methionine supplementation). Although no particular varietal differences were noted among the legumes without methionine supplementation, there were considerable differences evident when this gross deficiency was corrected. No doubt other essential amino acids also play a role in the biologic value of the legume proteins.

When 0.6 per cent methionine was included in the diet, nearly all the legumes (not soybeans or chick peas) showed a further gain, raising them to at least two thirds of the value of unsupplemented dried whole beef. In general, peas showed the least further gain, but the authors do not offer judgment as to whether this indicates a lesser deficiency of methionine in peas. Upon returning to the basal diet, the weight gains promptly returned to low levels, showing that there is no substantial body storage of methionine.

The growth gains did not show any relation to the amount of protein in the legume, but more important were not related to the methionine content of the protein as determined chemically. Thus although soybeans contained a relatively large amount of methionine, Laxtonian peas contained even more but gave only a fraction of the growth response of soybeans. Chick peas, with essentially the same methionine content as most of the peas and snap beans, were greatly superior. This apparent discrepancy with the methionine content is not due to inadequacy of other essential amino acids but rather to the availability of the methionine, since addition of crystalline methionine to the diet produces a much greater response than the protein methionine. As an example the authors cite that only one third of the protein methionine in the Laxtonian pea diet, when added as the pure amino acid, caused the large gain of 2.1 g., while the pea diet itself resulted in little growth. There is evidence that the methionine of soybeans

and chick peas is more available than that in the other legumes studied. These results make it doubtful whether purely chemical assays for amino acids can at all approximate biologic protein value.

As general conclusions it may be stated that peas, snap beans, and lima beans are relatively inadequate in protein value when used as the sole source of dietary protein, due in large part to unavailability of their methionine rather than their gross content; that their biologic value can be greatly enhanced and even approach that of meat with the addition of methionine; and that there are considerable varietal differences apparent in legume proteins when the diet contains added methionine.

THE INDISPENSABILITY OF ARGININE FOR MAXIMAL GROWTH OF THE RAT

As the result of years of effort in many laboratories it is now well established that nine of the naturally occurring amino acids are essential for the rat. The group which has probably contributed most definitively to this conclusion is that working under the direction of W. C. Rose at the University of Illinois. By using only mixtures of synthetic or highly purified amino acids this group was able to furnish unequivocal evidence as to the importance of each of nine amino acids for the nutrition of the rat. By 1942, it was possible for W. C. Rose and S. S. Fierke (*J. Biol. Chem.* **143,** *115* (*1942*)) to state that an additional nine amino acids were completely dispensable for the rat. This left four amino acids unclassified: viz., arginine, proline, hydroxyproline, and glutamic acid.

This laboratory has now presented clear evidence that arginine is required for maximal growth of the rat (A. Borman *et al.*, *J. Biol. Chem.* **166,** *585* (*1946*)). This seems at first surprising since C. W. Scull and W. C. Rose (*Ibid.* **89,** *109* (*1930*)) demonstrated the ability of the rat to synthesize arginine. The actual situation appears to be that although the rat can synthesize this amino acid it cannot make it fast enough for its needs during rapid growth. This explains the difficulty of demonstrating an arginine requirement in earlier studies, which were made before the ready availability of all the vitamins in highly purified form. In an effort to prevent the incorporation in the diet of significant amounts of unknown amino acids possibly present in the then crude vitamin supplements, only suboptimal amounts of certain of the vitamins were available, and hence growth was never maximal. Thus, in one preliminary experiment, control rats without arginine gained an average of 29 g. in twenty-eight days, whereas with added arginine they gained only 36 g., a statistically valid but not impressive enhancement of growth.

With the advent of abundant pure vitamin preparations, these studies were repeated. Twenty-eight rats were divided into three groups and placed on a basal ration adequately supplemented with the known vitamins and containing the recognized nine dispensable and nine indispensable amino acids, but free of arginine, proline, hydroxyproline, and glutamic acid. One group served as controls and gained an average of 39.0 g. during the twenty-eight day experiment. The second group, given a supplement of 0.24 g. of l-arginine monohydrochloride per 100 g. of diet, gained an average of 72.6 g. during the same interval—nearly twice as much as the controls. A third group supplemented with twice the equivalent amount of arginic acid (alpha-hydroxy analogue of arginine) gained only 47.2 g., a meager increase suggesting that arginic acid is only converted to arginine to the extent of 8 or 10 per cent.

Arginine is, therefore, indispensable for

maximal growth in the rat as it is in the chicken. Each animal species seems completely incapable of synthesizing certain nutrients, and can, therefore, not grow or even survive unless supplied with these indispensable compounds. A second group of nutrients, typified in this instance by arginine, can be synthesized, but at a rate insufficient to meet the needs of rapid growth. As investigation turns from the basic needs for survival to the requirements for "optimal" nutrition, this second group of nutrients becomes of increasing interest.

ANTITHIAMINE ACTIVITY OF PLANT MATERIALS

Horses and cattle which consume large amounts of fern (Pteris aquilina) often become ill of "fern poisoning." In connection with investigations of an outbreak of "fern poisoning," P. H. Weswig, A. M. Freed, and J. R. Haag (*J. Biol. Chem.* **165,** *737 (1946)*) have discovered what appears to be not only a lead to the cause of the disease but also a new means of producing thiamine deficiency.

Rats fed rations containing 40 per cent ground, air-dried fern and an estimated 0.2 to 0.6 mg. of thiamine per 100 g. of ration gained weight for ten days, after which they failed and died at about twenty days with anorexia, emaciation, and what appeared to be polyneuritis. That thiamine deficiency polyneuritis was involved was confirmed by the fact that 0.5 mg. of thiamine daily *per os* led to rapid loss of symptoms and prompt gain in weight of treated animals, while the controls invariably died. The rats receiving the supplementary thiamine made good gains over a five week period, thus indicating that rations containing 40 per cent fern were otherwise innocuous.

The mode of action of the "antithiamine" effect of the dehydrated fern is under investigation. Several possibilities come to mind. The fern might contain an enzyme or other substances capable of destroying the thiamine in the diet before consumption in the mixed ration, or in the stomach or intestinal tract. If this were true, the situation would be analogous to that found with the flesh of carp and certain other fish which contains an enzyme able to destroy the thiamine in foods with which it comes in contact (*Nutrition Reviews* **2,** *128 (1944)*). In the fern, however, an enzyme does not appear to be involved, since the activity is not decreased in air-dried fern heated to 105° for eighteen hours. Nonenzymatic thiamine-destroying factors have been reported to be present in certain seeds (*Ibid.* **4,** *222 (1946)*) and could perhaps explain the findings in fern poisoning. Another possibility would be the presence of a substance in the fern with a chemical structure so similar to that of thiamine that it could successfully compete with thiamine for the active enzyme centers in the cells in the sense that para-aminobenzoic acid competes with the "sulfa" drugs. Other examples of this competitive inhibitory type of action have been reviewed by D. W. Woolley (*Advances in Enzymology* **6,** *129 (1946)*).

The possibility must also be considered that a hypothetical material present in the fern might inhibit some other step in a chain of reactions in which thiamine functions, thus blocking the use of this vitamin by the cells. Finally, it is conceivable that the fern might contain a substance which combines with thiamine, thus rendering it nonabsorbable just as avidin prevents the absorption of biotin (*Nutrition Reviews* **2,** *291 (1944)*). Whatever the explanation, it will be important to elucidate the mechanism and thereby to add to the knowledge of secondary factors which can influence the nutritive requirements.

HEMOLYTIC ANEMIA OF CHOLINE AND FAT ADMINISTRATION

Production of a hemolytic anemia is one of the reported complications of intravenous administration of fat for nutritional purposes (*Nutrition Reviews* **3,** *3 (1945)*). Such an anemia is evident even with excessively high fat diets (A. Loewy, L. W. Freeman, A. Marchello, and V. Johnson, *Am. J. Physiol.* **138,** *230 (1942)*), but is not a general result of the even moderately high fat diet. The free fatty acids and soaps which escape resynthesis into neutral fat are thought to be responsible for the erythrocyte destruction. Addition of choline to a high fat diet produces marked destruction of erythrocytes in dogs, and choline itself, without excessive fat intake, slowly produces a hyperchromic anemia (*Nutrition Reviews* **4,** *83 (1946)*). Interpretation of the marked anemia-producing effect of choline and fat is difficult because the effective quantities of choline are comparable only to the amounts of choline required for prevention of fatty liver.

Several instances are now recognized in which nutritional essentials may exercise separate pharmacologic effects. The experiments of J. E. Davis (*Science* **105,** *43 (1947)*) indicate that the anemia production by choline is actually due to its known vasodilator action, rather than any nutritional effect. The drug atropine had been shown previously (*Am. J. Physiol.* **142,** *213, 402 (1944)*) to abolish or prevent the slowly progressing anemia of choline administration, hence its action seemed more likely to be related to the vasodilator action of choline than to lipotropic effects.

In the later experiments in which both choline and fat were administered, one splenectomized and 2 normal dogs were given 60 g. of fat and 10 mg. per kilogram of choline chloride daily in addition to a regular adequate diet. Two of the dogs were given in addition, after the first twenty-four hours, 0.5 mg. per kilogram of atropine sulfate daily. Erythrocyte and hemoglobin determinations were made daily. Despite the small number of animals employed, all showed a consistent drop of the erythrocyte count from over 6 million red blood cells to less than 5 million within the first twenty-four hours. The 2 dogs (one splenectomized) which then received atropine showed an immediate rise of the erythrocyte count to normal levels within the next forty-eight hours, while the dog not so treated reached even lower erythrocyte levels. Upon removing fat and choline from the diet the latter dog quickly regained a normal erythrocyte count.

Choline, through its vasodilator action, was concluded, therefore, to exert an inhibitory action on erythropoiesis in the bone marrow. In the absence of added choline normal erythropoiesis is presumed to compensate for erythrocyte destruction. It would seem necessary to assume that the choline normally present in the diet is readily assimilated to prohibit pharmacologically active concentrations in the blood.

The postulated action of improved blood and oxygen supply to the bone marrow in depressing erythropoiesis is of interest to the general problem of respiration and growth. The specific results provide a means of differentiating the nutritional and pharmacologic action of choline and emphasize the need for considering the pharmacologic action of administered vitamins.

EFFECTS OF IODINE INTAKE ON THYROXINE AND PLASMA IODINE LEVELS

A. Taurog and I. L. Chaikoff (*J. Biol. Chem.* **165,** *217 (1946)*) have studied in rats the influence of dietary iodine on the amounts of thyroxine and total iodine in the thyroid gland, and of total and protein-bound iodine in the plasma.

Adult rats which had been maintained on a diet containing 68.5 per cent wheat, 5 per cent casein, 10 per cent fish meal, 10 per cent alfalfa, 1.5 per cent sodium chloride, and 5 per cent sardilene (a fish oil containing 400 A.O.A.C. units of vitamin D and 3000 U.S.P. units of vitamin A per gram) were separated into seven groups. One group was continued on this same diet, which was shown to contain 0.3 microgram of iodine per gram. All other groups received a basal diet containing less iodine, one of them without added iodine, and the other five with the addition of potassium iodide supplying respectively 1, 5, 10, 20, and 30 micrograms of iodine per gram of diet. This basal diet contained 67.5 per cent whole wheat, 15 per cent casein, 10 per cent whole milk powder, 1 per cent sodium chloride, 1.5 per cent chalk, and 5 per cent hydrogenated vegetable oil, with the addition of 6.25 ml. of sardilene per kilogram of diet. This diet contained only 0.1 microgram of iodine per gram of diet.

Distilled water was given to the rats, so that the food was the only source of iodine, and each rat was kept in a separate metabolism cage so that its food consumption could be measured for purposes of calculating the iodine intake. The rats in the groups receiving diets of increasing iodine content ingested the following amounts of iodine (in micrograms) per day: 1 to 2, 3 to 6, 15 $\pm$ 0.6, 78 $\pm$ 2.7, 145 $\pm$ 7.2, 310 $\pm$ 18, and 440 $\pm$ 1.0, respectively. Each of these diets was fed for twenty-two to twenty-four days, and the rats were prepared for analysis of the thyroid and of the plasma by being fasted for twenty-four hours, at the end of which time the blood was removed and the thyroid excised. Pooled samples of plasma from 2 rats were used to obtain enough material for both total and protein-bound iodine determinations on the same sample, and occasionally the thyroids of 2 rats were pooled. Except in the two groups receiving the highest iodine intake, there were 8 or 14 rats in each group; a total of 70 male rats was used.

The results obtained showed that as the iodine intake increased, the thyroxine iodine of the thyroid rose to a maximum of about 44 mg. per 100 g. of fresh tissue at an intake of about 78 micrograms of iodine daily, and at greater intakes declined to 36.5 mg. per 100 g. of fresh tissue. The total iodine content of the gland also rose with increasing intake to a maximum value of about 140 mg. per 100 g. of fresh tissue at an intake of 145 micrograms of iodine daily, but showed a smaller drop as the intake increased further, to about 130 mg. per 100 g. of fresh tissue at the highest intake. Assay of the blood plasmas showed that as the iodine intake was raised from 1 to 2 micrograms daily to 78 micrograms daily, the level of protein-bound iodine rose from 3.1 $\pm$ 0.1 to 5.0 $\pm$ 0.14 micrograms per 100 ml., but a further rise in the level of this constituent was not observed with further increases in intake. The total iodine of the plasma on the other hand increased up to the highest intake levels tested, and was 33.6 $\pm$ 2.5 micrograms of iodine per 100 ml. at an intake of 440 micrograms of iodine daily. The experimental points on the graph showing the relation of total plasma iodine to daily iodine intake suggest a linear relationship.

These results establish for the first time the dependence of both the total iodine and the protein-bound iodine in both the blood plasma and the thyroid gland of the rat upon the iodine consumption. Presumably the earlier observations that the iodine content of plasma and thyroid were independent must have been incorrect, due to the use of insufficiently delicate methods of analysis.

It is of interest that in the group of rats

ingesting 145 micrograms of iodine daily, the plasma contained 13.5 micrograms per cent of total iodine, while the thyroid contained 138 mg. per cent wet weight of the gland, indicating a concentrating power of 10,000 times under conditions that promoted maximal storage. It is also interesting that in the gland the fraction of total iodine present as thyroxine remained relatively constant at 31 per cent in rats whose intake varied from 1 to 2 micrograms to 440 micrograms daily.

The authors concluded that in the normal rat the level of the protein-bound iodine of the plasma was dependent on the thyroxine content of the thyroid and limited by the gland's capacity to produce thyroxine.

STUDIES IN IRON METABOLISM

There are many gaps in our knowledge of iron metabolism (*Nutrition Reviews* **4,** *291 (1946)*). Thus, information concerning iron absorption has been based in part on the use of the radioactive iron technic. By this means P. F. Hahn, W. F. Bale, E. O. Lawrence, and G. H. Whipple (*J. Exp. Med.* **69,** *739 (1939)*) measured the amount of radioactive iron which appeared in the total circulating blood within five to seven days after oral administration of the isotope. The ratio of this value to the total quantity of tagged iron ingested was taken as a measure of the iron absorbed. In the review already referred to it was pointed out that conclusions concerning iron absorption cannot be drawn without considering various important limiting factors. A source of error involved in the radioactive iron method described above is clearly demonstrated by a study by R. Dubach, C. V. Moore, and V. Minnich (*J. Lab. Clin. Med.* **31,** *1201 (1946)*).

A patient who had all of the classic clinical and hematologic manifestations of pernicious anemia in relapse was given 1.0 mg. of radioactive iron per kilogram of body weight orally as ferrous chloride. Only about 1 per cent of the dose appeared in the peripheral hemoglobin during the next nine days. This might have been interpreted as indicating that the patient had absorbed only a very small percentage of the iron given. That another interpretation is the correct one was indicated by the changes which took place after liver extract was injected. On the ninth day liver extract was given and there followed the usual reticulocytosis, clinical improvement, and subsequent rise in both red blood cell and hemoglobin levels. Coincident with the rise in hemoglobin, additional amounts of radioactive iron made their appearance in the blood stream. Eventually more than 20 per cent of the administered oral dose was found in the circulating hemoglobin. Similar results were observed on other patients. Thus it was clear that the iron had been absorbed but could not be utilized until red cell formation was made possible by the administration of liver extract. When red cell formation became active the radioiron which presumably had gone to the stores in the interval was incorporated in the newly formed red cells.

Additional observations are described concerning the fate of radioactive iron administered intravenously. In 4 healthy young men 9 to 18 mg. of iron given intravenously in single doses as radioactive ferrous ascorbate appeared in the red cells to the extent of 60 to 70 per cent by the sixth day following injection. After this rapid utilization the amount in the red cells increased slowly until 80 per cent or more was present at the end of seven to ten weeks. In dogs, radioiron administered parenterally was used less completely and somewhat more slowly than in the healthy human subjects. From 0.2 to 9.0 mg. of labile iron as ferrous chloride or as ferrous ascorbate were injected into 13 animals. The total amount of the isotope which eventually appeared in the peripheral

blood of these animals varied from 36 to 72 per cent of the amount injected. In no instance was utilization as complete as in the human subjects. The completeness of utilization was not closely correlated with the size of the dose although the rate of iron utilization was roughly proportional to the per kilogram size of the dose.

The prompt utilization of the injected iron in normal human beings and dogs during the first week points to a peculiarity of iron metabolism. If the injected iron had been added to the whole of the body's store of nonhemoglobin iron and had been used at the same rate as the stored iron was being mobilized, then it would have appeared much more slowly as newly synthesized hemoglobin. The observations which are recorded would seem to indicate that newly injected iron is selectively used initially, at least, for new hemoglobin formation. Iron which has been stored for a longer period of time may not be so readily mobilized. This conclusion is consistent with those of J. F. Ross (*J. Clin. Invest.* **25,** *933 (1946)*) and of G. R. Greenberg and M. M. Wintrobe (*J. Biol. Chem.* **165,** *397 (1946)*). The latter investigators have referred to the existence of a "labile iron pool" made up of recently absorbed iron and of iron recently liberated from destroyed red cells from which the body takes the amount normally needed each day.

Dubach and her associates also attempted to determine what happens to the radioactive iron which does not appear in the peripheral blood as hemoglobin. The tissues of dogs killed after the injection of radioactive iron and then perfused with Locke's solution, were assayed for radioiron. In 2 animals 35.5 and 42.2 per cent of the radioiron was found in the blood and perfusion fluid, 10.3 and 1.8 per cent in the bone marrows of the respective dogs, 23.8 and 8.4 per cent in the liver, 4.2 and 3.3 per cent in the muscle, and smaller quantities in the range of 1 per cent in the spleen, kidneys, skin, and other tissues. In one animal 8.3 per cent was excreted during the experiment. The total recoveries were only 78 and 71.9 per cent, respectively. Where the "lost iron" goes is unknown although it is admitted that it may simply reflect inadequacies in the method of extraction of iron from the tissues and in the technic of measuring the isotope.

So far as the appearance of radioiron in the red cells is concerned, the importance of the factor of utilization is clearly demonstrated by these studies. Thus, in patients and dogs with iron deficiency the rate of uptake of intravenously injected radioactive iron was very rapid, and in the human subjects the uptake was practically 100 per cent. On the other hand, in patients with hypoplastic anemia in whom red cell formation was greatly impaired, the uptake of a similar quantity of radioiron was practically nil. In patients and in dogs with anemia due to blood destruction the uptake of radioactive iron was extremely irregular. The amounts of radioactive iron present in the peripheral blood at any one time varied widely from 10 to 90 per cent of the dose.

Returning to the question of the use of the radioactive iron method as a means for measuring iron absorption from the gastrointestinal tract, it becomes evident that this technic is accurate in subjects with uncomplicated iron deficiency in whom complete utilization of all iron finding its way into the blood plasma can be expected. In normal adult men the method is also reasonably satisfactory since the uptake by the red cells within six to nine days represents 70 to 90 per cent of the dose given. When used to study iron absorption in the presence of anemias in which blood formation is at a low ebb or in which blood destruction is occurring, the method gives misleading results.

PATHOGEN DIFFERENCES AND NATURAL RESISTANCE TO INFECTION

By *a priori* reasoning one may conclude that the nutritional state of the host plays a significant role in determining resistance or susceptibility to infection. It is difficult, however, to demonstrate such effects experimentally or to ascribe unequivocally the effects observed to specific nutrients. The experimental methods used and the observations recorded by a number of investigators in this field have been described previously in this journal (*Nutrition Reviews* **1,** *298 (1943)*; **2,** *178 (1944)*; **3,** *179 (1945)*; **4,** *203 234 (1946)*; **5,** *63 (1947)*).

Using Salmonella enteriditis, an organism producing mouse typhoid, as a test system, Dr. Schneider at the Rockefeller Institute for Medical Research (H. A. Schneider and L. T. Webster, *J. Exp. Med.* **81,** *359 (1945)*) attempted to demonstrate an effect of diet in natural resistance to infection in W-Swiss mice. Apparently well aware of the many environmental factors and characteristics of host and pathogen which might act to condition the results of his experiments, he painstakingly attempted to effect rigid control of as many such factors as was possible. The mice used in these experiments were derived from a colony which had been pen-inbred for seven years and included several strains of more highly inbred animals which had been demonstrated to be either relatively resistant or susceptible to infection by S. enteriditis. The mice were kept in specially designed cages in an air conditioned room with careful control of temperature, humidity, and lighting. Infection was induced by introduction through oral routes of 0.25 ml. of a dilution of an eighteen hour culture of S. enteriditis in meat infusion broth, calculated to contain the desired number of bacterial cells and checked by plate counts. The mice were observed daily after infection for thirty days, deaths recorded, and the survival rates determined. The data recorded were subjected to statistical analysis.

Under these conditions it was demonstrated that mice receiving a "natural" diet were more resistant to mouse typhoid than those receiving a so called "synthetic" diet in which all components were defined (*Nutrition Reviews* **3,** *215 (1945)*). Survival rates were 25 per cent higher in the animals receiving the "natural" diet. The factor responsible for this relative protection was found to be contained in whole wheat. Evidence was cited that neither protein nor known water soluble vitamins present in wheat were likely to be responsible for this effect.

Increased resistance to infection by S. enteriditis was reproducible in groups of W-Swiss mice receiving the diet containing whole wheat, if the mice were derived from strains which were genetically heterogeneous. However, this effect could not be demonstrated in mice which by strict inbreeding had been made more nearly homozygous and which were selected for use in the experiment because of their resistance or susceptibility to infection by S. enteriditis.

In a later paper (H. A. Schneider, *J. Exp. Med.* **84,** *305 (1946)*) further studies are reported in which the character of the bacterial population of the inoculum is described as important in demonstrating this effect of a component of the diet on resistance to infection. A single culture of S. enteriditis had been used as the source of infective material in earlier investigations. When in the course of these studies this culture on agar plates seemed to contain contaminants, it was subjected to mouse passage. The material obtained was again cultured on agar plates and a single colony which was characteristic in appearance was selected for culture in broth. This culture was then used to infect groups of mice on the "natural" and "synthetic" diets but no difference in survival rates was apparent. It seemed, therefore, that mouse passage and the picking of a single bacterial colony had so changed the conditions of the experi-

ment that an effect which previously had been readily reproducible no longer was observed. This organism had been subjected to repeated mouse passage and cultured in artificial mediums for many years in this laboratory without change in virulence or cultural characteristics. The author considered that the inability to reproduce the dietary effect of whole wheat on resistance under the conditions of this experiment was not due to the sudden appearance of genetic variation of the bacterium but rather to simplification of the bacterial population, since all cells included in the inoculum were recent descendants of a common cell. In contrast the original culture represented a heterogenetic bacterial population. To test this thesis a new culture of S. enteriditis was obtained from a healthy mouse carrier and was cultured so that an array of bacterial population was assured. The effect of the factor in whole wheat was again demonstrable when this newly isolated culture was used as pathogen. However, the use of a culture of this organism derived from a single colony again was ineffective in establishing conditions which would permit the demonstration of the nutritional effect on infection. These observations did not preclude the possibility that certain unique cells in the bacterial mass were responsible for providing the conditions essential for this effect. To rule out such a contingency it would have been necessary to test individually the 100,000 bacterial cells in the inoculum, a seemingly stupendous task.

It was possible to reproduce the earlier observations by assembling another bacterial population which provided the desired conditions. To do this another pathogen was used, S. typhimurium, which is immunologically distinct from S. enteriditis but which produces a similar disease in mice. Two variants of this organism were available, one virulent, the other avirulent to mice. The inclusion of whole wheat in the diet failed to change significantly the survival rates of mice infected with either bacterial strain individually. However, when the avirulent and virulent strains were combined in the infecting dose, the dietary effect on resistance was observed and was of the same magnitude as in earlier experiments with S. enteriditis. By this method an array of bacterial population had been provided. It is pointed out that although the two strains of S. typhimurium were selected because they exhibited differences in virulence, it is possible that characteristics other than the variation in virulence may be responsible for potentiating the difference in survival on the two diets.

Thus in this particular host-pathogen model it was shown that to demonstrate an effect of diet it was necessary to have a host population which was genetically heterogeneous and a bacterial culture of varied composition. The usual laboratory procedure is to attempt to work with homozygous hosts and "pure" cultures of pathogens. The conditions essential for the dietary effect observed here are precisely those which exist in nature. If this were a general phenomenon it would imply that the importance of nutrition in determining resistance to infection is most likely effective under those conditions which prevail in the world at large.

The nature of the substance in whole wheat which is effective in increasing the survival rates of mice to mouse typhoid has not yet been ascertained. It is of interest to note that other recent observations have demonstrated that there is in whole wheat a factor which is active in decreasing the resistance of mice to pneumococcal infections (G. H. Hitchings and E. A. Falco, *Science* **104,** *568 (1946)*).

There is no information to indicate how the factor contained in whole wheat is effective in increasing resistance of mice to Salmonella infections. J. W. Gowen (*Ann. Mo. Bot. Gardens* **32,** *187 (1945)*) who has worked with the same host-pathogen model, reported that mice of strains genetically

constituted so that they are particularly resistant to S. typhimurium maintain higher leucocyte counts and a greater proportion of white pulp in the spleen than do mice of susceptible strains. It would be of interest to determine if the factor in whole wheat mediates its effect through the reticulo-endothelial systems and if humoral mechanisms are involved.

The host-pathogen relationship is one which is complex and diverse. To elucidate fully the effects of nutritional factors on this relationship will test the ingenuities of immunologists and nutritionists alike.

RELATIONSHIP BETWEEN PLANE OF NUTRITION AND BIOCHEMICAL CAPABILITIES OF MICROORGANISMS

The intimate relationship which exists between the growth-factor requirements of microorganisms and the vitamin requirements of man has been mentioned frequently in this journal (*Nutrition Reviews* **4,** *153* (*1946*)). Such similarity lends to results of studies on nutrition of microorganisms more than passing interest to the animal nutritionist.

A number of illustrations of the effect of what may be called the "plane of nutrition" on the biochemical potentialities of microorganisms have appeared. L. J. Wickerham (*J. Bact.* **52,** *293* (*1946*)) has investigated the relationship between the vitamin content of the medium and the ability of yeasts to utilize various nitrogenous compounds as their sole supply of nitrogen for growth. In the past, the ability of yeasts to grow with potassium nitrate, ammonium sulfate, urea, asparagine, or peptone as the sole nitrogen source on a medium containing glucose and mineral salts has been used as an aid in their classification. While most yeasts, for example, can assimilate peptone under these conditions, the ability to utilize the other nitrogen sources listed is not universal, but varies greatly with different species and genera of yeasts. Wickerham now finds, however, that if adequate amounts of biotin, calcium pantothenate, inositol, niacin, para-aminobenzoic acid, pyridoxine, thiamine, and riboflavin are present in the medium, all yeasts tested were able to utilize each of these nitrogen compounds for growth. What previously appeared as variable ability to utilize individual nitrogen compounds for growth now appears to be a reflection of the variable ability of yeasts to synthesize the vitamins required for utilization of these nitrogen compounds.

J. R. Stockton and O. Wyss (*J. Bact.* **52,** *227* (*1946*)) report great variation in the amount of proteinase production by Bacillus subtilis in mediums containing different commercially available peptones and beef extracts, although little difference in growth on the same mediums was evident. The deficiency was traced to the ash content of some products. Addition of extra manganous ion resulted in good proteinase production on all mediums. More Mn^{++} was thus required for optimal proteinase production than was required for optimal growth. This recalls the work of W. D. Bellamy and I. C. Gunsalus (*Ibid.* **48,** *191* (*1944*)) who found that for production of maximum amounts of tyrosine decarboxylase, Streptococcus faecalis required much larger amounts of niacin and of vitamin B_6 than were required for maximum growth. They suggested that this was a case of "luxury consumption" of specific nutrients for the optimum function of cells. W. W. Umbreit and I. C. Gunsalus (*Ibid.* **50,** *124* (*1945*)) later showed that this same type of "luxury consumption" may occur in animals, for when rats were fed graded levels of pyridoxine, the amount of codecarboxylase present increased with the pyridoxine consumption up to 50 micrograms of pyridoxine

per day, whereas maximum growth was obtained with only 15 micrograms per day.

What profit, if any, organisms derive from the presence of increased amounts of specific systems over the minimum required for growth is not always apparent. That such variation can occur as a result of intake of specific nutrients in amounts greater than are necessary for maximum growth is of greatest interest, and should provide a fruitful field for further work.

THE DIGESTIBILITY OF FATS

The digestibility of fats has been studied rather extensively during the past thirty years but a large portion of the data has been collected by a limited group of workers using several types of experimental subjects. Among the first intensive studies on adult human beings were those made by C. F. Langworthy and A. D. Holmes in 1915 (*U. S. D. A. Bull.* **310** (*1915*)). The work of these two authors and of H. J. Deuel was later summarized by C. F. Langworthy (*Ind. Eng. Chem.* **15,** *276* (*1923*)). These workers reported the digestibilities of a wide variety of materials and hydrogenated fats. The digestibility of some of the more common animal and vegetable fats, shortenings, and several synthetic saturated triglycerides has been studied in albino rats by R. Hoagland and G. G. Snider (*U. S. D. A. Tech. Bull.* **725** (*1940*); *J. Nutrition* **26,** *219* (*1943*)). Another significant study was made by L. E. Holt, H. C. Tidwell, and C. M. Kirk (*Acta Paediat.* **16,** *165* (*1933*)) using human infants as experimental subjects.

These data have been subjected to correlation by K. F. Mattil (*Oil and Soap* **23,** *344* (*1946*)), who points out that it has been assumed that the coefficient of digestibility found for fat within any given experimental subject could be used to predict the digestibility of that fat in any other type of experimental subject. This assumption has never been established statistically and to do so Mattil assembled the data found in the literature and calculated the coefficient of correlation. He concluded that if two fats are equally digestible in rats they are probably equally so in human beings and if one fat is more digestible than another in one group it will probably be more digestible in the other.

Apparently fats as a class are well digested by almost any experimental subject, the majority of fats being more than 90 per cent assimilated. Most often in the literature there has been the suggestion that an inverse relationship exists between the digestibility of fats and their melting points. Despite numerous articles in the literature which indicate that this may not be true, the hypothesis has persisted.

Some factor other than the melting point supposedly determines the relative digestibility of these fats and there are considerable data to indicate that this factor may be associated with the stearic acid content. The term "stearic acid," as it is used here, refers to all saturated acids of eighteen or more carbon atoms. Stearic acid is almost entirely undigested by albino rats when fed as tristearin mixed with triolein (K. F. Mattil and J. W. Higgins, *J. Nutrition* **29,** *255* (*1945*)). In the study on human infants Holt and associates observed that individual fatty acids are selectively absorbed when a mixed fat is fed. It appears then that saturated acids of eighteen or more carbon atoms constitute the factor which limits the digestibility of fats. By analysis of all the available data Mattil has attempted to correlate statistically the stearic acid content of fats and their digestibility in human adults, human infants, and white rats. His studies seem to indicate that as the stearic acid content of

the fats increased their digestibility decreased. Human adults appear to metabolize a portion of the saturated acids of more than eighteen carbon atoms most readily, very few fats being less than 92 per cent digestible. Rats were found intermediate in their utilization of the long-chain saturated acids. The evidence indicated that human infants did not utilize completely any of the saturated acids but showed a marked preference for the unsaturated. A further study of the relationship of fats and their melting points revealed that the correlation which does exist between the digestibility of fats and their melting points depends on the effect of saturated acids on the melting points and is therefore merely a secondary effect. The primary factor which limits the digestibility of fats is the amount of saturated fatty acids present, and the degree of limitation increases as the length of the chain increases.

This study seems important in that it brings together the correlation of experimental studies of various types of subjects. It shows that at least in the digestion of fats, results in experimental animals coincide closely with those in human infants and adults.

THE LIFE SPAN OF THE RED BLOOD CELL

The measurement of the average life span of the red blood cell has been an intriguing challenge to physiologists and has occasioned much ingenious experimentation over the course of years. Although useful data were obtained, earlier attempts have never been accepted unreservedly since they all involved assumptions which were not completely acceptable. The methods were based on (a) the excretion of iron or red cell pigment, (b) the return to normal of the red cell count after artificially induced polycythemia, or (c) the disappearance of transfused red cells which had either been tagged with radioactive iron or were detectable because of immunologic differences from the red cells of the recipient. Although estimates thus obtained varied from five to two hundred days, more recent studies, freer from objection, gave values of eighty to one hundred twenty days for the average life span of the human red blood cell.

A new method has now been utilized to study this problem which seems to be free from previous objections. It is a tracer method based on the finding that glycine is one of the nitrogenous precursors of the protoporphyrin of hemoglobin (D. Shemin and D. Rittenberg, *J. Biol. Chem.* **159,** *567 (1945)*; **166,** *627 (1946)*). Before describing the red cell life span studies it may be useful to review a careful study on the rat confirming the utilization of glycine for hemoglobin formation (D. Shemin and D. Rittenberg, *Ibid.* **166,** *621 (1946)*). Rats on a protein free diet were fed glycine, glutamic acid, proline, leucine, or ammonia, each of which had been tagged with heavy nitrogen (N^{15}). The protein was withheld to prevent undue dilution of the isotopic amino acids. After two weeks the hemin was isolated and analyzed for N^{15}. The relative N^{15} concentration of the hemin was approximately 1, 2, and 10 respectively, from rats fed (a) ammonia or leucine, (b) glutamic acid or proline, and (c) glycine. Glycine is thus much better used for heme formation than the other forms of nitrogen. It is presumably used directly, whereas the nitrogen of other compounds is probably utilized indirectly via glycine. It is significant that the proline and glutamic acid were only slightly better utilized than ammonia, even though their structures have enough similarity to the pyrroles to make them seem logical heme precursors. The data now make this appear unlikely. In passing, it may be noted that in addition to these studies which indicate that part of the pyr-

role ring is formed from glycine, other studies from the same laboratory have shown that some of the carbons of the side-chains of heme are derived from acetate (K. Bloch and D. Rittenberg, *Ibid.* **159,** *45 (1945)*). Hence, a complete elaboration of the mechanism of heme formation is perhaps not too remote.

With the above information at hand, D. Shemin and D. Rittenberg (*J. Biol. Chem.* **166,** *627 (1946)*) fed to a human subject (the senior author) 66 g. of glycine which contained approximately one third of its nitrogen as N^{15}. The isotopic nitrogen in the hemin rose during the first eighteen days, then remained nearly constant until one hundred days had elapsed, and finally fell to low values during the subsequent one hundred and thirty days. The average life span was calculated to be one hundred and twenty-seven days which concurs with the recent values cited above, obtained by other methods. The concentration plateau which lasted for nearly one hundred days is especially interesting and indicates that the red cells formed during and shortly after the feeding of tagged glycine, persisted in the circulation without any significant destruction until they were nearly 100 days old and then began to breakdown fairly rapidly. This would seem to be an important observation and of interest to the general problem of aging. It shows for this special form of cell, at least, that the average life span is not merely the statistical result of exposure to a uniform hazard throughout the lifetime of each cell, but involves an actual period of aging before the particular cell becomes susceptible to destruction. These results may well stimulate investigation of the changes associated with or responsible for the aging of the red cell.

MICROBIOLOGIC SYNTHESIS OF RIBOFLAVIN

The ability of yeasts and other microorganisms to synthesize vitamins beyond their own nutritional needs is well known. With riboflavin, microbial production may be so great as to be of commercial significance. The vitamin is only slightly soluble in water, and may crystallize out of the growth medium in essentially pure form.

An early report of riboflavin synthesis by yeast was made by A. Guilliermond, M. Fontaine, and A. Raffy (*Compt. rend.* **201,** *1077 (1935)*) who noted that Ashbya gossypii elaborated the vitamin in very slight amounts. Later, A. Raffy and M. Fontaine (*Ibid.* **205,** *1005 (1937)*) reported the production of large quantities of riboflavin by the closely related yeast Eremothecium ashbyii when grown on agar.

Production of riboflavin has been found to be influenced markedly by the constituents of the growth medium, not only with yeast but also with bacteria and molds. Candida guilliermondia was found by P. R. Burkholder (*Proc. Nat. Acad. Sci.* **29,** *166 (1943)*; *Arch. Biochem.* **3,** *121 (1943)*) to produce 12.5 micrograms per milliliter of medium when 2 g. of ammonium sulfate per liter was the only source of nitrogen. Addition of 2 g. of asparagine and 0.8 g. of glycine per liter increased the yield to 47 micrograms per milliliter, although the yeast growth was relatively unaffected. With these amino acids in the medium, glucose, fructose, mannose, and sucrose permitted high riboflavin formation, but maltose, xylose, galactose, and several other carbohydrates resulted in low yields. Low concentrations of cyanide (2×10^{-4}M) produced a twofold increase in synthesis of the vitamin.

F. W. Tanner, C. Vojnovich, and J. M. Van Lanen (*Science* **101,** *180 (1945)*) who confirmed and extended these studies noted that riboflavin yields were consistently highest when the trace elements were omitted from Burkholder's medium, and that

this effect was due almost entirely to the iron salts. The optimum concentration of iron was extremely critical; when the level was kept below 10 micrograms per liter, C. guilliermondia and C. flareri produced up to 216 micrograms of riboflavin per milliliter, but synthesis dropped to about 10 micrograms per milliliter as the iron content was raised to 100 micrograms per liter.

Complete removal of iron from the medium lowered the riboflavin yield only slightly, but the yeast yield was approximately halved. The largest yeast crops were obtained at an iron level of 100 micrograms per liter. Similar, though less dramatic changes were produced in Torula yeast by J. C. Lewis (*Arch. Biochem.* **4,** *217* (*1944*)). A gossypii, on the other hand, did not seem to be so adversely affected by iron. L. J. Wickerham, M. H. Flickinger, and R. M. Johnston (*Ibid.* **9,** *95* (*1946*)) obtained up to 381 micrograms of riboflavin per milliliter with this yeast without taking precautions to remove iron from the medium.

The ability of A. gossypii to tolerate iron may be similar to that of Clostridium acetobutylicum. Tanner *et al.* found that the optimum iron level for the latter organism was approximately 1 microgram per milliliter, which is one hundred times greater than the optimum for Candida.

The results with cyanide seem reasonable in view of the iron effect, since cyanide is known to block iron-containing respiratory mechanisms. These mechanisms are dealt with in a study of riboflavin inhibition in Cl. acetobutylicum by A. Leviton (*J. Am. Chem. Soc.* **68,** *835* (*1946*)) who observed that not only was riboflavin production inhibited, but added riboflavin was destroyed in a medium containing ferrous ion and hydrogen peroxide, although the vitamin was unaffected by either reagent separately. The rate of destruction was characteristic of a unimolecular reaction, increasing from 2.3 per cent to 18.9 per cent per hour as the ferrous ion concentration rose from 100 micrograms to 300 micrograms per milliliter.

Although the presence of hydrogen peroxide could not be demonstrated in Cl. acetobutylicum, it was assumed that it was decomposed during fermentation. Since riboflavin-containing coenzymes are capable of forming hydrogen peroxide, this supposition seems reasonable. Moreover, it was found that 7 micrograms per milliliter of catalase permitted an increase in riboflavin production from 7 to 18 micrograms per milliliter in the presence of 10 micrograms of ferrous ion per milliliter. Finally, the reducing agent sodium hydrosulfite permitted an increase from 0.8 to 2 micrograms per milliliter when the iron concentration was near its critical point. This point was about 20 micrograms of ferrous ion per milliliter. Above this level, catalase became relatively ineffective in maintaining riboflavin production, and it was inferred that the destruction was an intracellular process, while catalase functioned outside the cell. In the presence of high concentrations of iron, destruction presumably took place within the cell before the vitamin (and the hydrogen peroxide) could diffuse into the catalase-containing medium. Low iron levels, on the other hand, would result in low peroxide formation, and diffusion could take place before excessive destruction of riboflavin occurred.

Ferric iron was completely inactive in reducing riboflavin synthesis, and it was assumed that any product formed with riboflavin must be highly dissociable. The nature of the ferrous-riboflavin product was not investigated.

These papers help to provide a background for an understanding of this interesting synthesis. Further studies should elucidate the role and the nature of the ferrous-riboflavin complex. It would also be of interest to know whether the relatively low production of this vitamin by most organisms is due to lack of synthesis or to accelerated destruction.

NOTES

Letters to the Editor

Dear Sir:

Reference is made to the review, "Utilization of Homocystine for Growth by the Rat" which appeared in *Nutrition Reviews* **5,** *13* (*1947*). Mention is made therein of the work of A. White and E. F. Beach (*J. Biol. Chem.* **122,** *219* (*1937*)) in which rats were studied in methionine deficiency produced by the use of peanut globulin, arachin, as the source of dietary protein. The following errors were made in the *Nutrition Reviews* interpretation:

1. *Nutrition Reviews*' article states, "The diet was incapable of supporting good growth and that the nutritional inadequacy of arachin was due to its indigestibility, as had been previously claimed." This is contrary to the statement in the conclusion of the original paper, "The nutritional inadequacy of arachin is attributable to its low methionine content rather than to indigestibility."

2. *Nutrition Reviews* then states with reference to the principal conclusions, "Although based on only 2 rats the results appear significant." This sheds doubt upon a piece of work which was actually carried out painstakingly on a total of 140 animals. The paper of White and Beach clearly states that the representative growth curves illustrate the findings on substantial numbers of animals and the numbers of animals used for each observation are given throughout the paper. Many animals were fed the basal arachin ration either unsupplemented or supplemented with methionine, cystine or homocystine. Readers who appreciate the importance of extensive control work in such animal studies will surely be misled by this misinterpretation which reflects discredit on a piece of work carefully planned and executed.

Abraham White
Eliot Beach
Children's Fund of Michigan
Detroit

Dear Sir:

I read with much interest the review article entitled "Present Knowledge of Vitamin D in Nutrition," which appeared in the February, 1947 issue of *Nutrition Reviews*. I am much concerned about the last phrase in the last sentence of the article.... "...and milk production has also been rendered more uniform." Aside from demonstrating that dairy cattle require vitamin D, workers in this field have not yet demonstrated that the feeding of vitamin D to milking cows will result in increased or sustained milk production.

W. E. Krauss
Ohio Agricultural
Experiment Station
Wooster, Ohio

Dear Sir:

As always I have read *Nutrition Reviews* with great interest and great appreciation. There is a statement in the article entitled "Present Knowledge of Vitamin D in Nutrition" which appears in the February issue which I would question and that is the comment concerning the action of vitamin D on the natural absorption of calcium and phosphate from the food and the manner in which it is exerted. I have read most of the papers on this subject and feel that there is no evidence warranting the statement that vitamin D directly increases the permeability of the intestinal mucosa to calcium salts while leaving unchanged the permeability of other membranes.

S. B. Wolbach
Harvard Medical School
Boston

World Food Appraisal for 1946–47

One of the activities of FAO is making World Food Appraisals (*Nutrition Reviews* **5,** *127* (*1947*)). The appraisal for 1946–47 was issued December 26, 1946 (*World Food Appraisal for 1946–47, Food and Agriculture Organization of the United Nations, Washington, U. S. A., December 26, 1946*). This

report emphasizes that although the world food situation is slightly better in 1946–47 than it was in 1945–46, a major food crisis still confronts the world. The primary cause of the continuation of the world's food crisis is attributed to the difficulty in recovering from the sharp wartime decline in agricultural production in Europe, the U. S. S. R., and the areas formerly occupied by Japan.

Riboflavin in Rat Muscle and Liver

Some of Dr. H. C. Sherman's earlier objectives on the effect of dietary levels of vitamin A over a long period on the organ content of the vitamin have been extended to riboflavin. A Z. Murray, L. M. Greenstein, and H. C. Sherman (*J. Biol. Chem.* **165,** *91 (1946)*) employed rats of both sexes which were closely related genetically and whose diets differed only by the addition of pure riboflavin to raise the dietary level of this factor. The riboflavin content of the large leg muscle and the liver of the rats was measured at various ages on the two diets.

The basal diet was an air-dried mixture of 5 parts of ground whole wheat and 1 part of dried whole milk, with added 2 per cent of sodium chloride. This diet supplied 3 to 3.5 micrograms of riboflavin per gram of food, and the second diet was fortified to supply 6 to 7 micrograms. The tissue riboflavin determination was a modification of the fluorometric method of A. Z. Hodson and L. C. Norris (*J. Biol. Chem.* **131,** *621 (1939)*) and particular care was taken to have uniformity in the method of removing and preparing the samples for analysis.

Significant differences in the riboflavin content of muscle were noted with advancing age. At 30 days of age (suggested as the "end of infancy") the fresh muscle contained 4.1 to 4.3 micrograms of riboflavin per gram of fresh weight, during the next 30 days dropped to about 3.5 micrograms, and at 300 to 540 days in the rats with the lower riboflavin diet contained only 2.2 to 2.4 micrograms. No differences were noted between the sexes.

The liver contained a larger but more variable amount of riboflavin. At 60 days the level was 25.4 to 28.4 micrograms per gram, with no significant difference due to sex or diet. With advancing age there was a slow increase in the riboflavin content of the liver, and a suggestion that the diet of higher riboflavin content resulted in a slightly higher level than the diet of lower riboflavin content.

Skim Cheese in Egypt

"Skim cheese" is an important item in the dietary of the poor in Egypt, and M. M. Taha El-Katib (*Nature* **158,** *747 (1946)*) points out its nutritive value. Although there are many variations in its preparation, the protein content ranged from 6.7 to 21.9 per cent, with an average of 16.6 per cent. The fat content was as high as 19.5 per cent in samples to which whole milk had been added. The calcium content ranged from 0.13 to 0.82 per cent, and phosphorus from 0.11 to 0.57 per cent. Valuable amounts of niacin, but not of thiamine, were present. Nutritionally, the product resembles the cottage cheese of England and America. The digestibility of protein, fat, calcium, and phosphorus in this product was favorable, with evidence that the presence of fat increases the utilization of the protein and phosphorus.

The author regards skim cheese, because of its low cost, as an indispensable protective food of the poor in Egypt. Although it is deprived of the fat soluble vitamins, it contains much first-class protein, some of the vitamin B factors, and valuable minerals.

ERRATUM

Volume 5, page 58, February 1947, column 2, line 17 should read "which is the unnatural form."

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

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